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<p>(54) Title: LAUNDRY AND CLEANING COMPOSITIONS CONTAINING HEXOSAMINIDASE ENZYMES</p> <p>(57) Abstract</p> <p>Laundry or cleaning products comprising one or more hexosaminidase enzymes, and methods for laundering fabrics and cleaning dishes and tableware with aqueous solution containing an effective amount of one or more hexosaminidase enzymes.</p>		

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## LAUNDRY AND CLEANING COMPOSITIONS CONTAINING HEXOSAMINIDASE ENZYMES

### TECHNICAL FIELD

The present invention relates to laundry and cleaning compositions having antimicrobial activity comprising hexosaminidase enzymes.

### BACKGROUND OF THE INVENTION

Laundry and cleaning composition having antimicrobial activities are of interest to consumers. Efforts to formulate antimicrobial hand soaps and cleaning compositions are well known. Efforts to produce laundry compositions comprising enzymes having microbial properties are also known, for example, U.S. 5,356,803, issued October 18, 1994 to Carpenter et al.

In spite of such efforts, there continues to be a need for laundry and cleaning compositions having antimicrobial activity. An object of the invention is to provide laundry and cleaning compositions having antimicrobial activity containing hexosaminidase enzymes. These and other objects will be apparent from the detailed description herein.

### BACKGROUND ART

US 5,356,803 is directed to the use of Type II endoglycosidases (Endo-D, Endo-H, Endo-F and PNGaseF) in laundry and cleaning compositions. See also: US 5,258,304; US 5,395,541; J. Biol. Chem. (1996), 271 (52), 33425-33432; WO 96/25424; Nat. Struct. Biol. (1996), 3(7), 638-648; Microbiology (1994), 140 (12), 3399-3406; J. Bacteriol. (1994), 176(9), 2640-7; Proc. Nat'l Acad. Sci. USA (1993), 90(14), 6751-5; Proc. Natl. Acad. Sci. USA (1985), 82 (23), 7830-4; and WO 96/36700.

### SUMMARY OF THE INVENTION

The present invention relates to laundry or cleaning products comprising one or more hexosaminidase enzymes, preferably at a level of from about 0.001% to about 1%, more preferably from about 0.01% to about 0.5%, by weight of the composition. More preferred are hexosaminidases having minimum inhibitory concentration ("MIC") for antimicrobial activity of less than about 0.125%, most

preferably less than about 0.025%, and/or the ability to remove biofilm. The present invention also relates to a method for laundering fabrics (preferably clothes), said method comprising contacting fabrics in need of cleaning with an aqueous solution containing an effective amount of one or more hexosaminidase enzymes, preferably an aqueous solution of a composition according to the present invention. The present invention further relates to a method for cleaning hard surfaces, such as dishes and tableware, said method comprising contacting the hard surface in need of cleaning with an aqueous solution containing an effective amount of one or more hexosaminidase enzymes, preferably an aqueous solution of a composition according to the present invention, and more preferably for dishes and tableware in an automatic dishwashing machine.

As used herein, the term "hexosaminidase enzyme" means those enzymes whose activity is for the hydrolysis of terminal non-reducing N-acetyl-D-hexosamine residues in N-acetyl- $\beta$ -D-hexosaminides, thereby acting on N-acetylglucosides and N-acetylgalactosides, and are classified under the class of enzymes EC 3.2.1.52 (also known as " $\beta$ -N-acetylhexosaminidase"). N-Acetyl- $\beta$ -D-hexosaminidase is also referred to as "chitobiosidases" or "exochitinase" (see for example, WO 96/36700). Hexosaminidases are known, for example those enzymes having the amino acid SEQ. ID No. 1-5 and 10-11 are classified in the literature as hexosaminidases. Furthermore, DNA sequences encoding for hexosaminidases are known, for example those having the SEQ ID No. 6-9. Examples of such disclosures in the literature include: J. Biol. Chem. (1996), 271 (52), 33425-33432; WO 96/25424; Nat. Struct. Biol. (1996), 3(7), 638-648; Microbiology (1994), 140 (12), 3399-3406; J. Bacteriol. (1994), 176(9), 2640-7; Proc. Nat'l Acad. Sci. USA (1993), 90(14), 6751-5; Proc. Natl. Acad. Sci. USA (1985), 82 (23), 7830-4; and WO 96/36700. In addition, a commercially available hexosaminidase is "exo- $\beta$ -N-acetylglucosaminidase" sold by Boehringer. Specific N-acetyl- $\beta$ -D-hexosaminidases from Saccharomyces cerevisiae DSM No. 9944 or DSM 9945 are also described in WO 96/36700.

Thus, more specifically, the invention encompasses laundry and cleaning compositions comprising a hexosaminidase enzyme exhibiting antimicrobial activity, which enzyme:

i) is encoded by a DNA sequence comprising or included in at least one of the sequences of SEQ ID Nos 6-9, or a sequence homologous thereto encoding a hexosaminidase polypeptide,

ii) is immunologically reactive with an antibody raised against a highly purified hexosaminidase encoded by the DNA sequence defined in i), and is specific for hexosaminidase,

iii) is immunologically reactive with an antibody raised against a highly purified hexosaminidase having SEQ ID Nos 1-5, 10 or 11, and is specific for hexosaminidase, or

iv) is a hexosaminidase having SEQ ID Nos 1-5, 10 or 11, or a hexosaminidase polypeptide sequence homologous thereto.

The terms "homologue" and "homologous" as used herein indicate a polypeptide encoded by DNA which hybridizes to the same probe as the DNA coding for an hexosaminidase enzyme under certain specified conditions (such as presoaking in 5xSSC and prehybridizing for 1 h at -40°C in a solution of 5xSSC, 5xDenhardt's solution, and 50 µg of denatured sonicated calf thymus DNA, followed by hybridization in the same solution supplemented with 50 µCi 32-P-dCTP labelled probe for 18 h at -40°C and washing three times in 2xSSC, 0.2% SDS at 40°C for 30 minutes). More specifically, the term is intended to refer to a DNA sequence which is at least 70% homologous to any of SEQ ID Nos 6-9, or the DNA encoding for the hexosaminidases of SEQ ID Nos 1-5, 10 or 11 including at least 75%, at least 80%, at least 85%, at least 90% or even at least 95% with any of these sequences. The term is intended to include modifications of any of such DNA sequences, such as nucleotide substitutions which do not give rise to another amino acid sequence of the polypeptide encoded by the sequence, but which correspond to the codon usage of the host organism into which a DNA construct comprising any of the DNA sequences is introduced or nucleotide substitutions which do give rise to a different amino acid sequence and therefore, possibly, a different amino acid sequence and

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therefore, possibly, a different protein structure which might give rise to a hexosaminidase mutant with different properties than the native enzyme. Other examples of possible modifications are insertion of one or more nucleotides into the sequence, addition of one or more nucleotides at either end of the sequence, or deletion of one or more nucleotides at either end or within the sequence.

The term "biofilm" as used herein means irreversibly bound bacteria to a surface.

All parts, percentages and ratios used herein are expressed as percent weight unless otherwise specified. All documents cited are, in relevant part, incorporated herein by reference.

#### DETAILED DESCRIPTION OF THE INVENTION

##### Hexosaminidases:

Hexosaminidases have been identified herein as particularly useful for their cleaning and/antimicrobial properties in laundry and cleaning compositions.

A hexosaminidase enzyme useful in the present invention may be isolated by a general method involving:

- cloning, in suitable vectors, a DNA library from a selected species,
- transforming suitable host cells with said vectors,
- culturing the host cells under suitable conditions to express any enzyme of interest encoded by a clone in the DNA library, and
- screening for positive clones by determining any hexosaminidase activity of the enzyme produced by such clones.

The DNA sequence encoding for the desired hexosaminidase enzyme may subsequently be inserted into a recombinant expression vector. This may be any vector which may conveniently be subjected to recombinant DNA procedures, and the choice of vector will often depend on the host cell into which it is to be introduced. Thus, the vector may be an autonomously replicating vector, i.e. a vector which exists as an extrachromosomal entity, the replication of which is independent of chromosomal replication, e.g. a plasmid. Alternatively, the vector may be one which, when introduced into a host cell, is integrated into the host cell

genome and replicated together with the chromosome(s) into which it has been integrated.

In the vector, the DNA sequence encoding the hexosaminidase should be operably connected to a suitable promoter and terminator sequence. The promoter may be any DNA sequence which shows transcriptional activity in the host cell of choice and may be derived from genes encoding proteins either homologous or heterologous to the host cell. The procedures used to ligate the DNA sequences coding for the hexosaminidase, the promoter and the terminator, respectively, and to insert them into suitable vectors are well known to persons skilled in the art (cf., for instance, Sambrook et al., Molecular Cloning. A Laboratory Manual, Cold Spring Harbor, NY 1989).

The host cell which is transformed with the DNA sequence encoding the enzyme useful for the present invention compositions is preferably a eukaryotic cell, in particular a fungal cell such as a yeast or filamentous fungal cell. Fungal cells may be transformed by a process involving protoplast formation and transformation of the protoplasts followed by regeneration of the cell wall in a manner known in the art. The host cell may also be a yeast cell, e.g. a strain of *Saccharomyces*, in particular *Saccharomyces cerevisiae*.

The medium used to culture the transformed host cells may be any conventional medium suitable for growing the host cells in question. The expressed hexosaminidase may conveniently be secreted into the culture medium and may be recovered therefrom by well-known procedures including separating the cells from the medium by centrifugation or filtration, precipitating proteinaceous components of the medium by means of a salt such as ammonium sulphate, followed by chromatographic procedures such as ion exchange chromatography, affinity chromatography, or the like.

The thus purified hexosaminidase may be employed for immunization of animals for the production of antibodies. More specifically, antiserum against the hexosaminidase may be raised by immunizing rabbits (or other rodents) according to the procedure described by N. Axelsen et al. in: A Manual of Quantitative Immunoelectrophoresis, Blackwell Scientific Publications, 1973, Chapter 23, or A.

Johnstone and R. Thorpe, Immunochemistry in Practice, Blackwell Scientific Publications, 1982 (more specifically pp. 27-31). Purified immunoglobulins may be obtained from the antisera, for example by salt precipitation  $((\text{NH}_4)_2\text{SO}_4)$ , followed by dialysis and ion exchange chromatography, e.g. on DEAE-Sephadex.

Immunochemical characterization of proteins may be done either by Ouchterlony double-diffusion analysis (O. Ouchterlony in: Handbook of Experimental Immunology (D.M. Weir, Ed.), Blackwell Scientific Publications, 1967, pp. 655-706), by crossed immunoelectrophoresis (N. Axelsen et al., supra, Chapters 3 and 4), or by rocket immunoelectrophoresis (N. Axelsen et al., Chapter 2).

The enzyme preparation useful in the present invention compositions may be prepared in accordance with methods known in the art and may be in the form of a liquid or a dry preparation. For instance, the enzyme preparation may be in the form of a granulate or a microgranulate. The enzyme to be included in the preparation may also be stabilized in accordance with methods known in the art.

The enzyme preparation useful in the present compositions may, in addition to a hexosaminidase, contain one or more other detergent enzymes and/or other plant cell wall degrading enzymes, for instance those with cellulytic, xylanolytic or pectinolytic activities such as xylanase, arabinanase, rhamnogalacturonase, pectin acylesterase, galactanase, polygalacturonase, pectin lyase, pectate lyase, endoglucanase or pectin methylesterase. The additional enzyme(s) may be producible by means of a microorganism belonging to the genus *Aspergillus*, preferably *aspergillus niger*, *Aspergillus aculeatus*, *Aspergillus awamoi* or *Aspergillus oryzae*.

#### Test Methods:

The potency of antimicrobial activity of the hexosaminidase useful herein is measured by determining the minimum inhibitory concentration (MIC) of enzyme required to inhibit growth of bacteria/fungi. For example, the bacteria used can include *Escherichia coli* 25922, 11229, *Staphylococcus aureus* 25932, 6538, *Pseudomonas aeruginosa* 27853 and *Proteus mirabilis* 12453.

The minimum inhibitory concentration of enzyme to inhibit growth of bacteria is determined in Robbins Scientific 96 well microassay Microplates with 50  $\mu\text{l}$  wells. 105  $\mu\text{l}$  of stock solutions of the single bacteria (from ATCC) are diluted in



15 ml of growth medium based on Tryptic Soy Broth/Agar (Carr-Scarrborough). The enzyme samples are diluted to 8000 ppm active enzyme in buffer solution. 10  $\mu$ l of buffer is added to each well. 10  $\mu$ l of enzyme solution is added in the first well. The enzyme solution is diluted in subsequent wells by 50%, by sequential transfer of 10  $\mu$ l. After final dilution 10  $\mu$ l of bacteria with growth medium is added to each well. All manipulations are performed with sterile material. All plates are incubated at 37°C for 12-24 hours. The growth of bacteria is assessed under a microscope. The minimum inhibitory concentration is determined by the lowest enzyme concentration which does not show bacteria growth. Preferred hexosaminidases for use herein have antimicrobial activity of less than about 0.125%.

Scanning electron microscopy can be used to determine biofilm removal. Preferred hexosaminidases for use herein have the ability to remove biofilm.

#### Cleaning Composition Ingredients and Detergent Compositions

The detergent compositions of the invention contain laundry or cleaning composition ingredients as described hereinafter. The precise nature of these components, and levels of incorporation thereof will depend on the physical form of the composition, and the nature of the cleaning operation for which it is to be used.

The detergent compositions according to the invention can be liquid, paste, gels, bars, tablets, powder or granular forms. Granular compositions can also be in "compact" form, the liquid compositions can also be in a "concentrated" form.

The compositions of the invention may for example, be formulated as hand and machine laundry detergent compositions including laundry additive compositions and compositions suitable for use in the soaking and/or pretreatment of stained fabrics, rinse added fabric softener compositions. Pre-or post treatment of fabric include gel, spray and liquid fabric conditioning compositions.

When formulated as compositions suitable for use in a laundry machine washing method, the compositions of the invention preferably contain both a surfactant and a builder compound and additionally one or more detergent components preferably selected from organic polymeric compounds, bleaching agents, additional enzymes, suds suppressors, dispersants, lime-soap dispersants, soil

suspension and anti-redeposition agents and corrosion inhibitors. Laundry compositions can also contain softening agents, as additional detergent components.

The compositions of the invention can also be used as detergent additive products. Such additive products are intended to supplement or boost the performance of conventional detergent compositions.

If needed the density of the laundry detergent compositions herein ranges from 400 to 1200 g/litre, preferably 600 to 950 g/litre of composition measured at 20°C.

The "compact" form of the compositions herein is best reflected by density and, in terms of composition, by the amount of inorganic filler salt; inorganic filler salts are conventional ingredients of detergent compositions in powder form; in conventional detergent compositions, the filler salts are present in substantial amounts, typically 17-35% by weight of the total composition.

In the compact compositions, the filler salt is present in amounts not exceeding 15% of the total composition, preferably not exceeding 10%, most preferably not exceeding 5% by weight of the composition.

The inorganic filler salts, such as meant in the present compositions are selected from the alkali and alkaline-earth-metal salts of sulphates and chlorides. A preferred filler salt is sodium sulphate.

Liquid detergent compositions according to the present invention can also be in a "concentrated form", in such case, the liquid detergent compositions according the present invention will contain a lower amount of water, compared to conventional liquid detergents.

Typically the water content of the concentrated liquid detergent is preferably less than 40%, more preferably less than 30%, most preferably less than 20% by weight of the detergent composition.

#### Surfactants

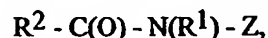
Preferably, the detergent compositions according to the present invention comprise a surfactant or surfactant system wherein the surfactant can be selected from nonionic and/or anionic and/or cationic and/or ampholytic and/or zwitterionic and/or semi-polar nonionic surfactants.

The surfactant is typically present at a level of from 0.1% to 60% by weight. More preferred levels of incorporation are 1% to 35% by weight, most preferably from 1% to 30% by weight of detergent compositions in accord with the invention.

The surfactant is preferably formulated to be compatible with enzyme components present in the composition. In liquid or gel compositions the surfactant is most preferably formulated such that it promotes, or at least does not degrade, the stability of any enzyme in these compositions.

Examples of suitable nonionic, anionic, cationic, ampholytic, zwitterionic and semi-polar nonionic surfactants are disclosed in U.S. Patent Nos. 5,707,950 and 5,576,282.

Highly preferred nonionic surfactants are polyhydroxy fatty acid amide surfactants of the formula:



wherein  $R^1$  is H, or  $R^1$  is  $C_{1-4}$  hydrocarbonyl, 2-hydroxy ethyl, 2-hydroxy propyl or a mixture thereof,  $R^2$  is  $C_{5-31}$  hydrocarbonyl, and Z is a polyhydroxyhydrocarbonyl having a linear hydrocarbonyl chain with at least 3 hydroxyls directly connected to the chain, or an alkoxylated derivative thereof. Preferably,  $R^1$  is methyl,  $R^2$  is a straight  $C_{11-15}$  alkyl or  $C_{16-18}$  alkyl or alkenyl chain such as coconut alkyl or mixtures thereof, and Z is derived from a reducing sugar such as glucose, fructose, maltose, lactose, in a reductive amination reaction.

Highly preferred anionic surfactants include alkyl alkoxylated sulfate surfactants hereof are water soluble salts or acids of the formula  $RO(A)_mSO_3M$  wherein R is an unsubstituted  $C_{10}-C_{24}$  alkyl or hydroxyalkyl group having a  $C_{10}-C_{24}$  alkyl component, preferably a  $C_{12}-C_{20}$  alkyl or hydroxyalkyl, more preferably  $C_{12}-C_{18}$  alkyl or hydroxyalkyl, A is an ethoxy or propoxy unit, m is greater than zero, typically between about 0.5 and about 6, more preferably between about 0.5 and about 3, and M is H or a cation which can be, for example, a metal cation (e.g., sodium, potassium, lithium, calcium, magnesium, etc.), ammonium or substituted-ammonium cation. Alkyl ethoxylated sulfates as well as alkyl propoxylated sulfates are contemplated herein.

When included therein, the laundry detergent compositions of the present invention typically comprise from about 1% to about 40%, preferably from about 3% to about 20% by weight of such anionic surfactants.

Highly preferred cationic surfactants are the water-soluble quaternary ammonium compounds useful in the present composition having the formula :



wherein  $R_1$  is  $C_8-C_{16}$  alkyl, each of  $R_2$ ,  $R_3$  and  $R_4$  is independently  $C_1-C_4$  alkyl,  $C_1-C_4$  hydroxy alkyl, benzyl, and  $-(C_2H_4O)_xH$  where  $x$  has a value from 2 to 5, and  $X$  is an anion. Not more than one of  $R_2$ ,  $R_3$  or  $R_4$  should be benzyl.

When included therein, the detergent compositions of the present invention typically comprise from 0.2% to about 25%, preferably from about 1% to about 8% by weight of such cationic surfactants.

When included therein, the detergent compositions of the present invention typically comprise from 0.2% to about 15%, preferably from about 1% to about 10% by weight of such ampholytic surfactants.

When included therein, the detergent compositions of the present invention typically comprise from 0.2% to about 15%, preferably from about 1% to about 10% by weight of such zwitterionic surfactants.

When included therein, the detergent compositions of the present invention typically comprise from 0.2% to about 15%, preferably from about 1% to about 10% by weight of such semi-polar nonionic surfactants.

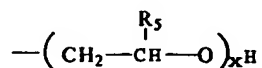
The detergent composition of the present invention may further comprise a cosurfactant selected from the group of primary or tertiary amines.

Suitable primary amines for use herein include amines according to the formula  $R_1NH_2$  wherein  $R_1$  is a  $C_6-C_{12}$ , preferably  $C_6-C_{10}$  alkyl chain or  $R_4X(CH_2)_n$ ,  $X$  is  $-O-$ ,  $-C(O)NH-$  or  $-NH-$ ,  $R_4$  is a  $C_6-C_{12}$  alkyl chain  $n$  is between 1 to 5, preferably 3.  $R_1$  alkyl chains may be straight or branched and may be interrupted with up to 12, preferably less than 5 ethylene oxide moieties.

Preferred amines according to the formula herein above are  $n$ -alkyl amines. Suitable amines for use herein may be selected from 1-hexylamine, 1-octylamine, 1-decylamine and laurylamine. Other preferred primary amines include  $C_8-C_{10}$

oxypropylamine, octyloxypropylamine, 2-ethylhexyl-oxypropylamine, lauryl amido propylamine and amido propylamine.

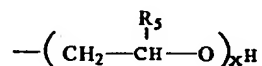
Suitable tertiary amines for use herein include tertiary amines having the formula  $R_1R_2R_3N$  wherein  $R_1$  and  $R_2$  are  $C_1$ - $C_8$  alkylchains or



$R_3$  is either a  $C_6$ - $C_{12}$ , preferably  $C_6$ - $C_{10}$  alkyl chain, or  $R_3$  is  $R_4X(\text{CH}_2)_n$ , whereby  $X$  is  $-\text{O}-$ ,  $-\text{C}(\text{O})\text{NH}-$  or  $-\text{NH}-$ ,  $R_4$  is a  $C_4$ - $C_{12}$ ,  $n$  is between 1 to 5, preferably 2-3.  $R_5$  is H or  $C_1$ - $C_2$  alkyl and  $x$  is between 1 to 6.

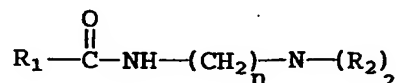
$R_3$  and  $R_4$  may be linear or branched;  $R_3$  alkyl chains may be interrupted with up to 12, preferably less than 5, ethylene oxide moieties.

Preferred tertiary amines are  $R_1R_2R_3N$  where  $R_1$  is a  $C_6$ - $C_{12}$  alkyl chain,  $R_2$  and  $R_3$  are  $C_1$ - $C_3$  alkyl or



where  $R_5$  is H or  $\text{CH}_3$  and  $x = 1-2$ .

Also preferred are the amidoamines of the formula:



wherein  $R_1$  is  $C_6$ - $C_{12}$  alkyl;  $n$  is 2-4,

preferably  $n$  is 3;  $R_2$  and  $R_3$  is  $C_1$ - $C_4$

Most preferred amines of the present invention include 1-octylamine, 1-hexylamine, 1-decylamine, 1-dodecylamine, C8-10oxypropylamine, N coco 1-3diaminopropane, coconutalkyldimethylamine, lauryldimethylamine, lauryl bis(hydroxyethyl)amine, coco bis(hydroxyethyl)amine, lauryl amine 2 moles propoxylated, octyl amine 2 moles propoxylated, lauryl amidopropyldimethylamine, C8-10 amidopropyldimethylamine and C10 amidopropyldimethylamine.

The most preferred amines for use in the compositions herein are 1-hexylamine, 1-octylamine, 1-decylamine, 1-dodecylamine. Especially desirable are n-dodecyldimethylamine and bishydroxyethylcoconutalkylamine and oleylamine 7

times ethoxylated, lauryl amido propylamine and cocoamido propylamine.

The surfactant and surfactant system of the present invention is preferably formulated to be compatible with enzyme components present in the composition. In liquid or gel compositions the surfactant is most preferably formulated such that it promotes, or at least does not degrade, the stability of any enzyme in these compositions.

#### Builders

The compositions according to the present invention may further comprise a builder or builder system. Any conventional builder system is suitable for use herein including aluminosilicate materials, silicates, polycarboxylates, alkyl- or alkenyl-succinic acid and fatty acids, materials such as ethylenediamine tetraacetate, diethylene triamine pentamethyleneacetate, metal ion sequestrants such as aminopolyposphonates, particularly ethylenediamine tetramethylene phosphonic acid and diethylene triamine pentamethylenephosphonic acid. Phosphate builders can also be used herein.

The present invention may include a suitable builder or detergency salt. The level of detergent salt/builder can vary widely depending upon the end use of the composition and its desired physical form. When present, the compositions will typically comprise at least about 1% builder and more typically from about 10% to about 80%, even more typically from about 15% to about 50% by weight, of the builder. Lower or higher levels, however, are not meant to be excluded.

Inorganic or P-containing detergent salts include, but are not limited to, the alkali metal, ammonium and alkanolammonium salts of polyphosphates (exemplified by the tripolyphosphates, pyrophosphates, and glassy polymeric metaphosphates), phosphonates, phytic acid, silicates, carbonates (including bicarbonates and sesquicarbonates), sulphates, and aluminosilicates. However, non-phosphate salts are required in some locales. Importantly, the compositions herein function surprisingly well even in the presence of the so-called "weak" builders (as compared with phosphates) such as citrate, or in the so-called "underbuilt" situation that may occur with zeolite or layered silicate builders.

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Organic detergent builders suitable for the purposes of the present invention

include, but are not restricted to, a wide variety of polycarboxylate compounds. As used herein, "polycarboxylate" refers to compounds having a plurality of carboxylate groups, preferably at least 3 carboxylates. Polycarboxylate builder can generally be added to the composition in acid form, but can also be added in the form of a neutralized salt. When utilized in salt form, alkali metals, such as sodium, potassium, and lithium, or alkanolammonium salts are preferred.

Examples of suitable silicate builders, carbonate salts, aluminosilicate builders, polycarboxylate builders, citrate builders, 3,3-dicarboxy-4-oxa-1,6-hexanedioate builders and related compounds disclosed in U.S. Patent No. 4,566,984, to Bush, succinic acid builders, phosphorous-based builders and fatty acids, are disclosed in U.S. Patent Nos. 5,576,282, 5,728,671 and 5,707,950.

Additional suitable builders can be an inorganic ion exchange material, commonly an inorganic hydrated aluminosilicate material, more particularly a hydrated synthetic zeolite such as hydrated zeolite A, X, B, HS or MAP.

Specific polycarboxylates suitable for the present invention are polycarboxylates containing one carboxy group include lactic acid, glycolic acid and ether derivatives thereof as disclosed in Belgian Patent Nos. 831,368, 821,369 and 821,370. Polycarboxylates containing two carboxy groups include the water-soluble salts of succinic acid, malonic acid, (ethylenedioxy) diacetic acid, maleic acid, diglycollic acid, tartaric acid, tartronic acid and fumaric acid, as well as the ether carboxylates described in German Offenlegenschrift 2,446,686, and 2,446,687 and U.S. Patent No. 3,935,257 and the sulfinyl carboxylates described in Belgian Patent No. 840,623. Polycarboxylates containing three carboxy groups include, in particular, water-soluble citrates, aconitrates and citraconates as well as succinate derivatives such as the carboxymethyloxysuccinates described in British Patent No. 1,379,241, lactoxysuccinates described in Netherlands Application 7205873, and the oxypolycarboxylate materials such as 2-oxa-1,1,3-propane tricarboxylates described in British Patent No. 1,387,447.

Polycarboxylates containing four carboxy groups include oxydisuccinates disclosed in British Patent No. 1,261,829, 1,1,2,2-ethane tetracarboxylates, 1,1,3,3-propane tetracarboxylates and 1,1,2,3-propane tetracarboxylates. Polycarboxylates

containing sulfo substituents include the sulfosuccinate derivatives disclosed in British Patent Nos. 1,398,421 and 1,398,422 and in U.S. Patent No. 3,936,448, and the sulfonated pyrolysed citrates described in British Patent No. 1,082,179, while polycarboxylates containing phosphone substituents are disclosed in British Patent No. 1,439,000.

Alicyclic and heterocyclic polycarboxylates include cyclopentane-cis,cis,cis-tetracarboxylates, cyclopentadienide pentacarboxylates, 2,3,4,5-tetrahydro-furan - cis, cis, cis-tetracarboxylates, 2,5-tetrahydro-furan -cis - dicarboxylates, 2,2,5,5-tetrahydrofuran - tetracarboxylates, 1,2,3,4,5,6-hexane -hexacarboxylates and carboxymethyl derivatives of polyhydric alcohols such as sorbitol, mannitol and xylitol. Aromatic poly-carboxylates include mellitic acid, pyromellitic acid and the phthalic acid derivatives disclosed in British Patent No. 1,425,343.

Of the above, the preferred polycarboxylates are hydroxycarboxylates containing up to three carboxy groups per molecule, more particularly citrates.

Preferred builder systems for use in the present compositions include a mixture of a water-insoluble aluminosilicate builder such as zeolite A or of a layered silicate (SKS-6), and a water-soluble carboxylate chelating agent such as citric acid.

Preferred builder systems include a mixture of a water-insoluble aluminosilicate builder such as zeolite A, and a watersoluble carboxylate chelating agent such as citric acid. Preferred builder systems for use in liquid detergent compositions of the present invention are soaps and polycarboxylates.

Other suitable water-soluble organic salts are the homo- or co-polymeric acids or their salts, in which the polycarboxylic acid comprises at least two carboxyl radicals separated from each other by not more than two carbon atoms. Polymers of this type are disclosed in GB-A-1,596,756. Examples of such salts are polyacrylates of MW 2000-5000 and their copolymers with maleic anhydride, such copolymers having a molecular weight of from 20,000 to 70,000, especially about 40,000.

Detergency builder salts are normally included in amounts of from 5% to 80% by weight of the composition preferably from 10% to 70% and most usually from 30% to 60% by weight.

Bleaching agent



Additional optional detergent ingredients that can be included in the detergent compositions of the present invention include bleaching agents such as hydrogen peroxide, PB1, PB4 and percarbonate with a particle size of 400-800 microns. These bleaching agent components can include one or more oxygen bleaching agents and, depending upon the bleaching agent chosen, one or more bleach activators. When present oxygen bleaching compounds will typically be present at levels of from about 1% to about 25%.

The bleaching agent component for use herein can be any of the bleaching agents useful for detergent compositions including oxygen bleaches as well as others known in the art. The bleaching agent suitable for the present invention can be an activated or non-activated bleaching agent.

Examples of suitable bleaching agents are disclosed in U.S. Patent Nos. 5,707,950 and 5,576,282.

The hydrogen peroxide releasing agents can be used in combination with, for example, the bleach activators disclosed in U.S. Patent No. 5,707,950 or Phenolsulfonate ester of N-nonanoyl-6-aminocaproic acid (NACA-OBS, described in WO94/28106), which are perhydrolyzed to form a peracid as the active bleaching species, leading to improved bleaching effect. Also suitable activators are acylated citrate esters.

Useful bleaching agents, including peroxyacids and bleaching systems comprising bleach activators and peroxygen bleaching compounds for use in detergent compositions according to the invention are described in WO95/27772, WO95/27773, WO95/27774, WO95/27775 and U.S. Patent No. 5,707,950.

Metal-containing catalysts for use in bleach compositions, include cobalt-containing catalysts such as Pentaamine acetate cobalt(III) salts and manganese-containing catalysts such as those described in EPA 549 271; EPA 549 272; EPA 458 397; US 5,246,621; EPA 458 398; US 5,194,416 and US 5,114,611. Bleaching composition comprising a peroxy compound, a manganese-containing bleach catalyst and a chelating agent is described in the patent application No 94870206.3.

#### Dye transfer inhibition

The detergent compositions of the present invention can also include

compounds for inhibiting dye transfer from one fabric to another of solubilized and suspended dyes encountered during fabric laundering and conditioning operations involving colored fabrics.

*Polymeric dye transfer inhibiting agents*

The detergent compositions according to the present invention can also comprise from 0.001% to 10 %, preferably from 0.01% to 2%, more preferably from 0.05% to 1% by weight of polymeric dye transfer inhibiting agents. Said polymeric dye transfer inhibiting agents are normally incorporated into detergent compositions in order to inhibit the transfer of dyes from colored fabrics onto fabrics washed therewith. These polymers have the ability to complex or adsorb the fugitive dyes washed out of dyed fabrics before the dyes have the opportunity to become attached to other articles in the wash.

Especially suitable polymeric dye transfer inhibiting agents are polyamine N-oxide polymers, copolymers of N-vinylpyrrolidone and N-vinylimidazole, polyvinylpyrrolidone polymers, polyvinylloxazolidones and polyvinylimidazoles or mixtures thereof. Examples of such dye transfer inhibiting agents are disclosed in U.S. Patent Nos. 5,707,950 and 5,707,951.

Additional suitable dye transfer inhibiting agents include, but are not limited to, cross-linked polymers. Cross-linked polymers are polymers whose backbone are interconnected to a certain degree; these links can be of chemical or physical nature, possibly with active groups in the backbone or on branches; cross-linked polymers have been described in the Journal of Polymer Science, volume 22, pages 1035-1039.

In one embodiment, the cross-linked polymers are made in such a way that they form a three-dimensional rigid structure, which can entrap dyes in the pores formed by the three-dimensional structure. In another embodiment, the cross-linked polymers entrap the dyes by swelling.

Such cross-linked polymers are described in the co-pending European patent application 94870213.9

Addition of such polymers also enhances the performance of the enzymes according to the invention.

### Dispersants

The detergent composition of the present invention can also contain dispersants. Suitable water-soluble organic salts are the homo- or co-polymeric acids or their salts, in which the polycarboxylic acid comprises at least two carboxyl radicals separated from each other by not more than two carbon atoms.

Polymers of this type are disclosed in GB-A-1,596,756. Examples of such salts are polyacrylates of MW 2000-5000 and their copolymers with maleic anhydride, such copolymers having a molecular weight of from 1,000 to 100,000.

Especially, copolymer of acrylate and methylacrylate such as the 480N having a molecular weight of 4000, at a level from 0.5-20% by weight of composition can be added in the detergent compositions of the present invention.

The compositions of the invention may contain a lime soap peptiser compound, which has a lime soap dispersing power (LSDP), as defined hereinafter of no more than 8, preferably no more than 7, most preferably no more than 6. The lime soap peptiser compound is preferably present at a level from 0% to 20% by weight.

A numerical measure of the effectiveness of a lime soap peptiser is given by the lime soap dispersant power (LSDP) which is determined using the lime soap dispersant test as described in an article by H.C. Borghetty and C.A. Bergman, J. Am. Oil. Chem. Soc., volume 27, pages 88-90, (1950). This lime soap dispersion test method is widely used by practitioners in this art field being referred to, for example, in the following review articles; W.N. Linfield, Surfactant science Series, Volume 7, page 3; W.N. Linfield, Tenside surf. det., volume 27, pages 159-163, (1990); and M.K. Nagarajan, W.F. Masler, Cosmetics and Toiletries, volume 104, pages 71-73, (1989). The LSDP is the % weight ratio of dispersing agent to sodium oleate required to disperse the lime soap deposits formed by 0.025g of sodium oleate in 30ml of water of 333ppm  $\text{CaCO}_3$  (Ca:Mg=3:2) equivalent hardness.

Surfactants having good lime soap peptiser capability will include certain amine oxides, betaines, sulfobetaines, alkyl ethoxysulfates and ethoxylated alcohols.

Exemplary surfactants having a LSDP of no more than 8 for use in accord with the present invention include  $\text{C}_{16}$ - $\text{C}_{18}$  dimethyl amine oxide,  $\text{C}_{12}$ - $\text{C}_{18}$  alkyl

ethoxysulfates with an average degree of ethoxylation of from 1-5, particularly C<sub>12</sub>-C<sub>15</sub> alkyl ethoxysulfate surfactant with a degree of ethoxylation of amount 3 (LSDP=4), and the C<sub>14</sub>-C<sub>15</sub> ethoxylated alcohols with an average degree of ethoxylation of either 12 (LSDP=6) or 30, sold under the tradenames Lutensol A012 and Lutensol A030 respectively, by BASF GmbH.

Polymeric lime soap peptisers suitable for use herein are described in the article by M.K. Nagarajan, W.F. Masler, to be found in *Cosmetics and Toiletries*, volume 104, pages 71-73, (1989).

Hydrophobic bleaches such as 4-[N-octanoyl-6-aminohexanoyl]benzene sulfonate, 4-[N-nonanoyl-6-aminohexanoyl]benzene sulfonate, 4-[N-decanoyl-6-aminohexanoyl]benzene sulfonate and mixtures thereof; and nonanoyloxy benzene sulfonate together with hydrophilic / hydrophobic bleach formulations can also be used as lime soap peptisers compounds.

Examples of other suitable dispersing agents are disclosed in U.S. Patent Nos. 5,576,282 and 5,728,671.

#### Conventional detergent enzymes

The detergent compositions can comprise in addition to the hexosaminidase enzyme one or more enzymes which provide cleaning performance and/or fabric care benefits.

Said enzymes include enzymes selected from hemicellulases, peroxidases, proteases, cellulases, xylanases, lipases, phospholipases, esterases, cutinases, pectinases, keratanases, reductases, oxidases, phenoloxidases, lipxygenases, ligninases, pullulanases, tannases, pentosanases, malanases,  $\beta$ -glucanases, arabinosidases, hyaluronidase, chondroitinase, laccase, and known amylases, or mixtures thereof.

Examples of suitable enzymes are disclosed in U.S. Patent Nos. 5,576,282, 5,728,671 and 5,707,950

A preferred combination is a detergent composition having cocktail of conventional applicable enzymes like protease, lipase, cutinase and/or cellulase in conjunction with the hexosaminidase.

Particularly useful proteases are described in PCT publications: WO

95/30010 published November 9, 1995 by The Procter & Gamble Company; WO 95/30011 published November 9, 1995 by The Procter & Gamble Company; and WO 95/29979 published November 9, 1995 by The Procter & Gamble Company.

In addition to the peroxidase enzymes disclosed in U.S. Patent Nos. 5,576,282, 5,728,671 and 5,707,950, other suitable peroxidase enzymes are disclosed in European Patent application EP No. 96870013.8, filed February 20, 1996. Also suitable is the laccase enzyme.

Preferred enhancers are substituted phenothiazine and phenoxazine 10-Phenothiazinepropionic acid (PPT), 10-ethylphenothiazine-4-carboxylic acid (EPC), 10-phenoxazinepropionic acid (POP) and 10-methylphenoxazine (described in WO 94/12621) and substituted syringates (C3-C5 substituted alkyl syringates) and phenols. Sodium percarbonate or perborate are preferred sources of hydrogen peroxide.

Said peroxidases are normally incorporated in the detergent composition at levels from 0.0001% to 2% of active enzyme by weight of the detergent composition.

Other preferred enzymes that can be included in the detergent compositions of the present invention include lipases. Suitable lipase enzymes for detergent usage include those produced by microorganisms of the *Pseudomonas* group, such as *Pseudomonas stutzeri* ATCC 19.154, as disclosed in British Patent 1,372,034. Suitable lipases include those which show a positive immunological cross-reaction with the antibody of the lipase, produced by the microorganism *Pseudomonas fluorescent* IAM 1057. This lipase is available from Amano Pharmaceutical Co. Ltd., Nagoya, Japan, under the trade name Lipase P "Amano," hereinafter referred to as "Amano-P". Other suitable commercial lipases include Amano-CES, lipases ex *Chromobacter viscosum*, e.g. *Chromobacter viscosum* var. *lipolyticum* NRRLB 3673 from Toyo Jozo Co., Tagata, Japan; *Chromobacter viscosum* lipases from U.S. Biochemical Corp., U.S.A. and Disoynt Co., The Netherlands, and lipases ex *Pseudomonas gladioli*. Especially suitable lipases are lipases such as M1 Lipase<sup>R</sup> and Lipomax<sup>R</sup> (Gist-Brocades) and Lipolase<sup>R</sup> and Lipolase Ultra<sup>R</sup>(Novo) which have found to be very effective when used in combination with the compositions of

the present invention.

Also suitable are cutinases [EC 3.1.1.50] which can be considered as a special kind of lipase, namely lipases which do not require interfacial activation. Addition of cutinases to detergent compositions have been described in e.g. WO 88/09367 (Genencor).

The lipases and/or cutinases are normally incorporated in the detergent composition at levels from 0.0001% to 2% of active enzyme by weight of the detergent composition.

Known amylases ( $\alpha$  and/or  $\beta$ ) can be included for removal of carbohydrate-based stains. WO 94/02597, Novo Nordisk A/S published February 03, 1994, describes cleaning compositions which incorporate mutant amylases. See also WO94/18314, Genencor, published August 18, 1994 and WO95/10603, Novo Nordisk A/S, published April 20, 1995. Other amylases known for use in detergent compositions include both  $\alpha$ - and  $\beta$ -amylases.  $\alpha$ -Amylases are known in the art and include those disclosed in US Pat. 5,003,257; EP 252,666; WO 91/00353; FR 2,676,456; EP 285,123; EP 525,610; EP 368,341; and British Patent Specification No. 1,296,839 (Novo). Other suitable amylase are stability-enhanced amylases including Purafact Ox Am<sup>R</sup> described in WO 94/18314, published August 18, 1994 and WO96/05295, Genencor, published February 22, 1996 and amylase variants from Novo Nordisk A/S, disclosed in WO 95/10603, published April 95.

Examples of commercial  $\alpha$ -amylases products are Termamyl<sup>®</sup>, Ban<sup>®</sup>, Fungamyl<sup>®</sup> and Duramyl<sup>®</sup>, all available from Novo Nordisk A/S Denmark. WO95/26397 describes other suitable amylases :  $\alpha$ -amylases characterised by having a specific activity at least 25% higher than the specific activity of Termamyl<sup>®</sup> at a temperature range of 25°C to 55°C and at a pH value in the range of 8 to 10, measured by the Phadebas<sup>®</sup>  $\alpha$ -amylase activity assay. Other amylolytic enzymes with improved properties with respect to the activity level and the combination of thermostability and a higher activity level are described in WO95/35382.

The above-mentioned enzymes may be of any suitable origin, such as vegetable, animal, bacterial, fungal and yeast origin. Purified or non-purified forms of these enzymes may be used. Also included by definition, are mutants of native

enzymes. Mutants can be obtained e.g. by protein and/or genetic engineering, chemical and/or physical modifications of native enzymes. Common practice as well is the expression of the enzyme via host organisms in which the genetic material responsible for the production of the enzyme has been cloned.

Said enzymes are normally incorporated in the detergent composition at levels from 0.0001% to 2% of active enzyme by weight of the detergent composition. The enzymes can be added as separate single ingredients (prills, granulates, stabilized liquids, etc. containing one enzyme) or as mixtures of two or more enzymes (e.g. cogramulates).

Other suitable detergent ingredients that can be added are enzyme oxidation scavengers. Examples of such enzyme oxidation scavengers are ethoxylated tetraethylene polyamines.

A range of enzyme materials and means for their incorporation into synthetic detergent compositions is also disclosed in WO 9307263 and WO 9307260 to Genencor International, WO 8908694 to Novo, and U.S. 3,553,139, January 5, 1971 to McCarty et al. Enzymes are further disclosed in U.S. 4,101,457, Place et al, July 18, 1978, and in U.S. 4,507,219, Hughes, March 26, 1985. Enzyme materials useful for liquid detergent formulations, and their incorporation into such formulations, are disclosed in U.S. 4,261,868, Hora et al, April 14, 1981. Enzymes for use in detergents can be stabilized by various techniques. Enzyme stabilisation techniques are disclosed and exemplified in U.S. 3,600,319, August 17, 1971, Gedge et al, EP 199,405 and EP 200,586, October 29, 1986, Venegas. Enzyme stabilisation systems are also described, for example, in U.S. 3,519,570. A useful *Bacillus*, sp. AC13 giving proteases, xylanases and cellulases, is described in WO 9401532 to Novo.

#### Chelating Agents

The detergent compositions herein may also optionally contain one or more iron and/or manganese chelating agents. Such chelating agents can be selected from the group consisting of amino carboxylates, amino phosphonates, polyfunctionally-substituted aromatic chelating agents and mixtures therein, all as hereinafter defined. Without intending to be bound by theory, it is believed that the benefit of these materials is due in part to their exceptional ability to remove iron and manganese.

ions from washing solutions by formation of soluble chelates.

Examples of suitable chelating agents are disclosed in U.S. Patent No. 5,728,671.

The compositions herein may also contain water-soluble methyl glycine diacetic acid (MGDA) salts (or acid form) as a chelant or co-builder useful with, for example, insoluble builders such as zeolites, layered silicates and the like.

If utilized, these chelating agents will generally comprise from about 0.1% to about 15% by weight of the detergent compositions herein. More preferably, if utilized, the chelating agents will comprise from about 0.1% to about 3.0% by weight of such compositions.

#### Suds suppressor

Another optional ingredient is a suds suppressor, exemplified by silicones, and silica-silicone mixtures. Examples of suitable suds suppressors are disclosed in U.S. Patent Nos. 5,707,950 and 5,728,671. These suds suppressors are normally employed at levels of from 0.001% to 2% by weight of the composition, preferably from 0.01% to 1% by weight.

#### Softening agents

Fabric softening agents can also be incorporated into laundry detergent compositions in accordance with the present invention. These agents may be inorganic or organic in type. Inorganic softening agents are exemplified by the smectite clays disclosed in GB-A-1 400 898 and in USP 5,019,292. Organic fabric softening agents include the water insoluble tertiary amines as disclosed in GB-A1 514 276 and EP-B0 011 340 and their combination with mono C12-C14 quaternary ammonium salts are disclosed in EP-B-0 026 527 and EP-B-0 026 528 and di-long-chain amides as disclosed in EP-B-0 242 919. Other useful organic ingredients of fabric softening systems include high molecular weight polyethylene oxide materials as disclosed in EP-A-0 299 575 and 0 313 146.

Particularly suitable fabric softening agents are disclosed in U.S. Patent Nos. 5,707,950 and 5,728,673.

Levels of smectite clay are normally in the range from 2% to 20%, more preferably from 5% to 15% by weight, with the material being added as a dry mixed



component to the remainder of the formulation. Organic fabric softening agents such as the water-insoluble tertiary amines or dilong chain amide materials are incorporated at levels of from 0.5% to 5% by weight, normally from 1% to 3% by weight whilst the high molecular weight polyethylene oxide materials and the water soluble cationic materials are added at levels of from 0.1% to 2%, normally from 0.15% to 1.5% by weight. These materials are normally added to the spray dried portion of the composition, although in some instances it may be more convenient to add them as a dry mixed particulate, or spray them as molten liquid on to other solid components of the composition.

Typical cationic fabric softening components include the water-insoluble quaternary-ammonium fabric softening actives, the most commonly used having been di-long alkyl chain ammonium chloride or methyl sulfate.

Preferred cationic softeners among these include the following:

- 1) ditallow dimethylammonium chloride (DTDMAC);
- 2) dihydrogenated tallow dimethylammonium chloride;
- 3) dihydrogenated tallow dimethylammonium methylsulfate;
- 4) distearyl dimethylammonium chloride;
- 5) dioleyl dimethylammonium chloride;
- 6) dipalmityl hydroxyethyl methylammonium chloride;
- 7) stearyl benzyl dimethylammonium chloride;
- 8) tallow trimethylammonium chloride;
- 9) hydrogenated tallow trimethylammonium chloride;
- 10) C<sub>12-14</sub> alkyl hydroxyethyl dimethylammonium chloride;
- 11) C<sub>12-18</sub> alkyl dihydroxyethyl methylammonium chloride;
- 12) di(stearoyloxyethyl) dimethylammonium chloride (DSOEDMAC);
- 13) di(tallowoyloxyethyl) dimethylammonium chloride;
- 14) ditallow imidazolinium methylsulfate;
- 15) 1-(2-tallowylamidoethyl)-2-tallowyl imidazolinium methylsulfate.

Biodegradable quaternary ammonium compounds have been presented as alternatives to the traditionally used di-long alkyl chain ammonium chlorides and methyl sulfates. Such quaternary ammonium compounds contain long chain

alk(en)yl groups interrupted by functional groups such as carboxy groups. Said materials and fabric softening compositions containing them are disclosed in numerous publications such as EP-A-0,040,562, and EP-A-0,239,910.

Non-limiting examples of softener-compatible anions for the quaternary ammonium compounds and amine precursors include chloride or methyl sulfate.

#### Others

Other components used in detergent compositions may be employed, such as soil-suspending agents, soil-release agents, optical brighteners, abrasives, bactericides, tarnish inhibitors, coloring agents, and/or encapsulated or non-encapsulated perfumes, examples of which are disclosed in U.S. Patent Nos. 5,707,950, 5,576,282 and 5,728,671.

It is well known in the art that free chlorine in tap water rapidly deactivates the enzymes comprised in detergent compositions. Therefore, using chlorine scavenger such as perborate, ammonium sulfate, sodium sulphite or polyethyleneimine at a level above 0.1% by weight of total composition, in the formulas will provide improved through the wash stability of the detergent enzymes. Compositions comprising chlorine scavenger are described in the European patent application 92870018.6 filed January 31, 1992.

Alkoxylated polycarboxylates such as those prepared from polyacrylates are useful herein to provide additional grease removal performance. Such materials are described in WO 91/08281 and PCT 90/01815 at p. 4 et seq., incorporated herein by reference. Chemically, these materials comprise polyacrylates having one ethoxy side-chain per every 7-8 acrylate units. The side-chains are of the formula  $-(CH_2CH_2O)_m(CH_2)_nCH_3$  wherein m is 2-3 and n is 6-12. The side-chains are ester-linked to the polyacrylate "backbone" to provide a "comb" polymer type structure. The molecular weight can vary, but is typically in the range of about 2000 to about 50,000. Such alkoxylated polycarboxylates can comprise from about 0.05% to about 10%, by weight, of the compositions herein.

#### Method of washing

The compositions of the invention may be used in essentially any washing or cleaning methods, including soaking methods, pretreatment methods and methods

with rinsing steps for which a separate rinse aid composition may be added.

The process described herein comprises contacting fabrics with a laundering solution in the usual manner and exemplified hereunder.

The process of the invention is conveniently carried out in the course of the cleaning process. The method of cleaning is preferably carried out at 5°C to 95°C, especially between 10°C and 60°C. The pH of the treatment solution is preferably from 7 to 11.

The following examples are meant to exemplify compositions of the present invention, but are not necessarily meant to limit or otherwise define the scope of the invention. In the detergent compositions, the enzyme levels are expressed by pure enzyme by weight of the total composition and unless otherwise specified, the detergent ingredients are expressed by weight of the total compositions. The abbreviated component identifications herein have the following meanings:

LAS	: Sodium linear C <sub>12</sub> alkyl benzene sulphonate
TAS	: Sodium tallow alkyl sulphate
CXYAS	: Sodium C <sub>1X</sub> - C <sub>1Y</sub> alkyl sulfate
25EY	: A C <sub>12</sub> -C <sub>15</sub> predominantly linear primary alcohol condensed with an average of Y moles of ethylene oxide
CXYEZ	: A C <sub>1X</sub> - C <sub>1Y</sub> predominantly linear primary alcohol condensed with an average of Z moles of ethylene oxide
XYEZS	: C <sub>1X</sub> - C <sub>1Y</sub> sodium alkyl sulfate condensed with an average of Z moles of ethylene oxide per mole
QAS	: R <sub>2</sub> .N <sup>+</sup> (CH <sub>3</sub> ) <sub>2</sub> (C <sub>2</sub> H <sub>4</sub> OH) with R <sub>2</sub> = C <sub>12</sub> -C <sub>14</sub>

- Soap** : Sodium linear alkyl carboxylate derived from a 80/20 mixture of tallow and coconut oils.
- Nonionic** : C<sub>13</sub>-C<sub>15</sub> mixed ethoxylated/propoxylated fatty alcohol with an average degree of ethoxylation of 3.8 and an average degree of propoxylation of 4.5 sold under the tradename Plurafac LF404 by BASF GmbH.
- CFAA** : C<sub>12</sub>-C<sub>14</sub> alkyl N-methyl glucamide
- TFAA** : C<sub>16</sub>-C<sub>18</sub> alkyl N-methyl glucamide.
- TPKFA** : C<sub>12</sub>-C<sub>14</sub> topped whole cut fatty acids.
- DEQA** : Di-(tallow-oxy-ethyl) dimethyl ammonium chloride.
- Neodol 45-13** : C<sub>14</sub>-C<sub>15</sub> linear primary alcohol ethoxylate, sold by Shell Chemical CO.
- Silicate** : Amorphous Sodium Silicate (SiO<sub>2</sub>:Na<sub>2</sub>O ratio = 2.0)
- NaSKS-6** : Crystalline layered silicate of formula  $\delta$ -Na<sub>2</sub>Si<sub>2</sub>O<sub>5</sub>.
- Carbonate** : Anhydrous sodium carbonate with a particle size between 200  $\mu$ m and 900 $\mu$ m.
- Bicarbonate** : Anhydrous sodium bicarbonate with a particle size between 400  $\mu$ m and 1200 $\mu$ m.
- STPP** : Anhydrous sodium tripolyphosphate

- MA/AA** : Copolymer of 1:4 maleic/acrylic acid, average molecular weight about 70,000-80,000
- Zeolite A** : Hydrated Sodium Aluminosilicate of formula  $\text{Na}_{12}(\text{AlO}_2\text{SiO}_2)_{12} \cdot 27\text{H}_2\text{O}$  having a primary particle size in the range from 0.1 to 10 micrometers
- Citrate** : Tri-sodium citrate dihydrate of activity 86,4% with a particle size distribution between 425  $\mu\text{m}$  and 850  $\mu\text{m}$ .
- Citric** : Anhydrous citric acid
- PB1** : Anhydrous sodium perborate monohydrate bleach, empirical formula  $\text{NaBO}_2 \cdot \text{H}_2\text{O}_2$
- PB4** : Anhydrous sodium perborate tetrahydrate
- Percarbonate** : Anhydrous sodium percarbonate bleach of empirical formula  $2\text{Na}_2\text{CO}_3 \cdot 3\text{H}_2\text{O}_2$
- TAED** : Tetraacetyl ethylene diamine.
- NOBS** : Nonanoyloxybenzene sulfonate in the form of the sodium salt.
- Photoactivated Bleach** : Sulfonated zinc phthalocyanine encapsulated in dextrin soluble polymer.

- Protease** : Proteolytic enzyme sold under the tradename Savinase, Alcalase, Durazym by Novo Nordisk A/S, Maxacal, Maxapem sold by Gist-Brocades and proteases described in patents WO91/06637 and/or WO95/10591 and/or EP 251 446.
- Amylase** : Amylolytic enzyme sold under the tradename Purafact Ox Am<sup>R</sup> described in WO 94/18314, WO96/05295 sold by Genencor; Termamyl<sup>®</sup>, Fungamyl<sup>®</sup> and Duramyl<sup>®</sup>, all available from Novo Nordisk A/S and those described in WO95/26397.
- Lipase** : Lipolytic enzyme sold under the tradename Lipolase, Lipolase Ultra by Novo Nordisk A/S
- Hexosaminidase** : A hexosaminidase according to the present invention compositions, having MIC less than about 0.125%.
- Cellulase** : Cellulytic enzyme sold under the tradename Carezyme, Celluzyme and/or Endolase by Novo Nordisk A/S.
- CMC** : Sodium carboxymethyl cellulose.
- HEDP** : 1,1-hydroxyethane diphosphonic acid.
- DETPMP** : Diethylene triamine penta (methylene phosphonic acid), marketed by Monsanto under the Trade name Dequest 2060.
- PVNO** : Poly(4-vinylpyridine)-N-Oxide.
- PVPVI** : Poly (4-vinylpyridine)-N-oxide/copolymer of vinyl-imidazole and vinyl-pyrrolidone.

- Brightener 1 : Disodium 4,4'-bis(2-sulphostyryl)biphenyl.
- Brightener 2 : Disodium 4,4'-bis(4-anilino-6-morpholino-1.3.5-triazin-2-yl) stilbene-2:2'-disulfonate.
- Silicone antifoam : Polydimethylsiloxane foam controller with siloxane-oxyalkylene copolymer as dispersing agent with a ratio of said foam controller to said dispersing agent of 10:1 to 100:1.
- Granular Suds Suppressor : 12% Silicone/silica, 18% stearyl alcohol, 70% starch in granular form
- SRP 1 : Sulfobenzoyl or sodium isethionate end capped esters with oxyethylene oxy and terephthaloyl backbone.
- SRP 2 : Diethoxylated poly (1,2 propylene terephthalate) short block polymer.
- Sulphate : Anhydrous sodium sulphate.
- HMWPEO : High molecular weight polyethylene oxide

**Example 1**

The following detergent formulations, according to the present invention are prepared, where I and III are phosphorus-containing detergent compositions, and II is a zeolite-containing detergent composition:

	I	II	III
Blown Powder:			
STPP	24.0	-	24.0

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Zeolite A	-	24.0	-
C45AS	9.0	6.0	13.0
MA/AA	2.0	4.0	2.0
LAS	6.0	8.0	11.0
TAS	2.0	-	-
Silicate	7.0	3.0	3.0
CMC	1.0	1.0	0.5
Brightener 2	0.2	0.2	0.2
Soap	1.0	1.0	1.0
DETPMP	0.4	0.4	0.2
Spray On			
C45E7	2.5	2.5	2.0
C25E3	2.5	2.5	2.0
Silicone antifoam	0.3	0.3	0.3
Perfume	0.3	0.3	0.3
Dry additives:			
Carbonate	6.0	13.0	15.0
PB4	18.0	18.0	10.0
PB1	4.0	4.0	0
TAED	3.0	3.0	1.0
Photoactivated bleach	0.02	0.02	0.02
Protease	0.01	0.01	0.01
Lipase	0.009	0.009	-
Amylase	0.002	-	0.001
Hexosaminidase	0.05	0.01	0.001
Dry mixed sodium sulfate	3.0	3.0	5.0
Balance (Moisture &	100.0	100.0	100.0
Miscellaneous)			
Density (g/litre)	630	670	670

Example 2

The following nil bleach-containing detergent formulations of particular use in the



washing of colored clothing, according to the present invention are prepared:

	I	II	III
<b>Blown Powder</b>			
Zeolite A	15.0	15.0	-
Sodium sulfate	0.0	5.0	-
LAS	3.0	3.0	-
DETPMP	0.4	0.5	-
CMC	0.4	0.4	-
MA/AA	4.0	4.0	-
<b>Agglomerates</b>			
C45AS	-	-	11.0
LAS	6.0	5.0	-
TAS	3.0	2.0	-
Silicate	4.0	4.0	-
Zeolite A	10.0	15.0	13.0
CMC	-	-	0.5
MA/AA	-	-	2.0
Carbonate	9.0	7.0	7.0
<b>Spray On</b>			
Perfume	0.3	0.3	0.5
C45E7	4.0	4.0	4.0
C25E3	2.0	2.0	2.0
<b>Dry additives</b>			
MA/AA	-	-	3.0
NaSKS-6	-	-	12.0
Citrate	10.0	-	8.0
Bicarbonate	7.0	3.0	5.0
Carbonate	8.0	5.0	7.0
PVPV/PVNO	0.5	0.5	0.5
Protease	0.026	0.016	0.047
Lipase	0.009	-	0.009

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Amylase	0.005	0.005	--
Hexosaminidase	0.05	0.01	0.001
Cellulase	0.006	0.006	--
Silicone antifoam	5.0	5.0	5.0
Dry additives			
Sodium sulfate	0.0	9.0	0.0
Balance (Moisture and Miscellaneous)	100.0	100.0	100.0
Density (g/litre)	700	700	700

**Example 3**

The following detergent formulations, according to the present invention are prepared:

	<b>I</b>	<b>II</b>	<b>III</b>	<b>IV</b>
LAS	20.0	14.0	24.0	22.0
QAS	0.7	1.0	-	0.7
TFAA	-	1.0	-	-
C25E5/C45E7	-	2.0	-	0.5
C45E3S	-	2.5	-	-
STPP	30.0	18.0	30.0	22.0
Silicate	9.0	5.0	10.0	8.0
Carbonate	13.0	7.5	-	5.0
Bicarbonate	-	7.5	-	-
DETPMP	0.7	1.0	-	-
SRP 1	0.3	0.2	-	0.1
MA/AA	2.0	1.5	2.0	1.0
CMC	0.8	0.4	0.4	0.2
Hexosaminidase	0.05	0.01	0.001	0.05
Protease	0.008	0.01	0.026	0.026

Amylase	0.007	--	0.005	0.002
Lipase	0.004	--	--	0.002
Cellulase	0.0015	0.0005	-	-
Photoactivated bleach	70ppm	45ppm	-	10ppm
Brightener 1	0.2	0.2	0.08	0.2
PB1	6.0	2.0	-	-
NOBS	2.0	1.0	-	-
Balance (Moisture and Miscellaneous)	100	100	100	100

**Example 4**

The following liquid detergent formulations, according to the present invention are prepared:

	I	II	III	IV	V	VI	VII	VIII
LAS	10.0	13.0	9.0	-	25.0	-	-	-
C25AS	4.0	1.0	2.0	10.0	-	13.0	18.0	15.0
C25E3S	1.0	-	-	3.0	-	2.0	2.0	4.0
C25E7	6.0	8.0	13.0	2.5	-	-	4.0	4.0
TFAA	-	-	-	4.5	-	6.0	8.0	8.0
QAS	-	-	-	-	3.0	1.0	-	-
TPKFA	2.0	-	13.0	2.0	-	15.0	7.0	7.0
Rapeseed fatty acids	-	-	-	5.0	-	-	4.0	4.0
Citric	2.0	3.0	1.0	1.5	1.0	1.0	1.0	1.0
Dodecenyl/ tetradecenyl succinic acid	12.0	10.0	-	-	15.0	-	-	-
Oleic acid	4.0	2.0	1.0	-	1.0	-	-	-
Ethanol	4.0	4.0	7.0	2.0	7.0	2.0	3.0	2.0
1,2 Propanediol	4.0	4.0	2.0	7.0	6.0	8.0	10.0	13.-

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Mono Ethanol Amine	-	-	-	5.0	-	-	9.0	9.0
Tri Ethanol Amine	-	-	8	-	-	-	-	-
NaOH (pH)	8.0	8.0	7.6	7.7	8.0	7.5	8.0	8.2
Ethoxylated tetraethylene pentamine	0.5	-	0.5	0.2	-	-	0.4	0.3
DETPMP	1.0	1.0	0.5	1.0	2.0	1.2	1.0	-
SRP 2	0.3	-	0.3	0.1	-	-	0.2	0.1
PVNO	-	-	-	-	-	-	-	0.10
Hexosaminidase	0.05	0.01	0.001	0.05	0.01	0.001	0.05	0.05
Protease	.005	.005	.004	.003	0.08	.005	.003	.006
Lipase	-	.002	-	.0002	-	-	.003	.003
Amylase	.002	-	-	.004	.002	.008	.005	.005
Cellulase	-	-	-	.0001	-	-	.0004	.0004
Boric acid	0.1	0.2	-	2.0	1.0	1.5	2.5	2.5
Na formate	-	-	1.0	-	-	-	-	-
Ca chloride	-	0.015	-	0.01	-	-	-	-
Bentonite clay	-	-	-	-	4.0	4.0	-	-
Suspending clay	-	-	-	-	0.6	0.3	-	-
SD3								
Balance	100	100	100	100	100	100	100	100
Moisture and Miscellaneous								
<u>Example 5</u>								

Granular fabric detergent compositions which provide "softening through the wash" capability are prepared in accord with the present invention :

	I	II
45AS	-	10.0
LAS	7.6	-
68AS	1.3	-
45E7	4.0	-
25E3	-	5.0
Coco-alkyl-dimethyl hydroxy-ethyl ammonium chloride	1.4	1.0
Citrate	5.0	3.0
Na-SKS-6	-	11.0
Zeolite A	15.0	15.0
MA/AA	4.0	4.0
DETPMP	0.4	0.4
PB1	15.0	-
Percarbonate	-	15.0
TAED	5.0	5.0
Smectite clay	10.0	5.0
HMWPEO	-	0.1
Hexosaminidase	0.05	0.01
Protease	0.02	0.01
Lipase	0.02	0.01
Amylase	0.01	0.005
Cellulase	0.001	-
Silicate	3.0	5.0
Carbonate	10.0	10.0
Granular suds suppressor	1.0	4.0
CMC	0.2	0.1
Water/minors	Up to 100%	

**Example 6**

Syndet bar fabric detergent compositions are prepared in accord with the present invention :

	I	II	III	IV
C26 AS	20.00	20.00	20.00	20.00
CFAA	5.0	5.0	5.0	5.0
LAS (C11-13)	10.0	10.0	10.0	10.0
Sodium carbonate	25.0	25.0	25.0	25.0
Sodium pyrophosphate	7.0	7.0	7.0	7.0
STPP	7.0	7.0	7.0	7.0
Zeolite A	5.0	5.0	5.0	5.0
CMC	0.2	0.2	0.2	0.2
Polyacrylate (MW 1400)	0.2	0.2	0.2	0.2
Coconut monethanolamide	5.0	5.0	5.0	5.0
Hexosaminidase	0.05	0.01	0.001	0.05
Amylase	0.01	—	0.005	—
Protease	0.3	-	0.5	0.05
Brightener, perfume	0.2	0.2	0.2	0.2
CaSO <sub>4</sub>	1.0	1.0	1.0	1.0
MgSO <sub>4</sub>	1.0	1.0	1.0	1.0
Water	4.0	4.0	4.0	4.0
Filler* : balance to 100%				

\*Can be selected from convenient materials such as CaCO<sub>3</sub>, talc, clay (Kaolinite, Smectite), silicates, and the like.

#### Example 7

<u>Ingredients</u>	Weight %	
	A	B
STPP	24.0	45

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Sodium carbonate	20.0	13.5
Silicate	15.0	13.5
Nonionic surfactants	2.0	2.0
MA/AA	4.0	—
Protease	0.083	0.083
Amylase	0.005	0.005
Hexosaminidase	0.01	0.05
PB1	14.5	14.5
Cobalt catalyst*	0.008	—
Dibenzoyl peroxide (18% active)	4.4	4.4
Water, sodium sulfate and misc.	Balance	Balance

\*Pentaamineacetatocobalt (III) nitrate.

**Example 8**

Light-duty liquid dishwashing detergent formulae are prepared as follows:

Ingredient	Composition		
	A	B	C
	% Weight		
Surfactant	32.00	29.50	30.75
Ethanol	4.00	4.00	4.00
Ammonium citrate	0.06	0.06	0.06
Magnesium chloride	3.32	3.32	3.32
Ammonium sulfate	0.08	0.08	0.08
Hydrogen peroxide	200 ppm	—	—
Perfume	0.18	0.18	0.18
Protease	0.005	0.005	0.005
Amylase	0.005	0.005	0.005
Hexosaminidase	0.05	0.05	0.05
Water and minors	Balance	Balance	Balance

## SEQUENCE LISTING

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CONTAINING HEXOSAMINIDASE ENZYMES

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## (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

## (vi) CURRENT APPLICATION DATA:

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## (2) INFORMATION FOR SEQ ID NO:1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 611 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

1 MNYRIDFAVL SEHPQPCRFG LTIENLSDDQ LKAWSLHFTI DRYIQPDSIS  
51 HSQIHQVGSF CSLTPEQDVI NSNSHFYCEF SIKTAPFFPH YYTDGIKAAF  
101 VQINDVEPRV RHDVIVTPIA LASPYRERSE IPATDAATLS LLPKPNHIER  
151 LDGEFALTAG SQISLQSSCA ETAATWLKQE LTHLYQWQPH DIGSADIVLR



201 TNPTLDEGAY LLSVDRKPIR LEASSHIGFV HASATLLQLV RPDGDNLLVP  
 251 HIVIKDAPRF KYRGMMLDCA RHFHPLERVK RLINQLAHYK FNTFHWHLTD  
 301 DEGWRIEIKS LPQLTDIGAW RGVDEVLEPQ YSLLTEKHGG FYTQEEIREV  
 351 IAYAAERGIT VPEIDIPGH SRAAIKALPE WLFDEDDQSQ YRSIQYYNDN  
 401 VLSPALPGTY RFLDCVLKEV AALFPPSHFIH IGADEVDPDV WVNSPKCQAL  
 451 MAEGGYTDAK ELQGHLLRYA EKKLKSIGKR MVGWEEAQHG DKVSKDTVIY  
 501 SWLSEQAALN CARQGFDVIL QPGQFTYLDI AQDYAPEEPG VDWAGVTPLE  
 551 RAYRYEPLVE VPEHDP LRKR ILGIQCALWC ELVNNQDRMD YMIYPRLTAL  
 601 AGSGLDTKIP A

## 2) INFORMATION FOR SEQ ID NO: 2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 430 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

1 PRFPYRGIFL DVARNFHKKD AVLRLLDQMA AYKLNKPHFH LSDDEGWRIE  
 51 IPGLPELDEV GGQRCHDLSE TTCLLPQYQG GPDVYGGFFS RQDYIDIKY  
 101 AQARQIEVIP EIDMPAHARA AVVSMEARYK KLHAAGKEQE ANEFRLVDPT  
 151 DTSNTTSVQF FNRQSYLNPC LDSSQRFVDK VIGEIAQMHK EAGQPIKTWH  
 201 FGGDEARNIR LGAGYTDKAK PEPGKGIIDQ SNEDKPWAKS QVCQTMKEG  
 251 KVADMEHLPS YFGQEVSKLV KAHGIDRMOA WQDGLKDAES SKAFATSRVG  
 301 VNFWDITLYG GFDSVNDWAN KGYEVVSNP DYVYMDFFPYE VNPDERGYYY  
 351 GTRFSDERKV FSPAPDNMPQ NAETSVD RDG NHFNAKSDKP WPGAYGLSAQ  
 401 LWSETQRTDP QMEYMIFFRA LSAVERSWHR

## 2) INFORMATION FOR SEQ ID NO: 3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 777 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

1 MKRLTFGACI CCLLSLMACS QKAKQVQIPE YDKGINIPL PMQLTESDDS  
 51 FEVDDKTITC VSARELKPIA KLLADKLAS ADLSLQIEIG KEPSGNAIYI  
 101 GVD TALPLKE BGYMLRSDKR GVSIIKSAH GAFYGMQTL LQLPAEVES  
 151 NEVLLPMTVP GVEIKDEPAF GYRGFMLDVC RHFLSVEDIK KHIDIMAMFK  
 201 INRFHWHLTE DQAWRIEIKK YPRLTEVGST RTEGDGTQYS GFYTQEQVRD

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251 IVQYASDHFI TVIPMIEMPG HAMAALAAYP QFRCFPREFK PRIIWGVEQD  
 301 VYCAGKDSVF RFISDVIDEV APLFPPTYFH IGGDECPKDR WKACSLCQKR  
 351 MRDNGLKDEH ELQSYFIKQA EKVILQKHGKR LIGWDEILEG GLAPSATVMS  
 401 WRGEDGGIAA ANMNHVIMT PGSGGLYLDH YQGDPTVEPV AIGGYAPLEQ  
 451 VYAYNPLPKE LPADKHRYVL GAQANLWAEY LYTSERYDYQ AYPRLAVAE  
 501 LITWPLAKKD PADFCRRIDN ACVRIDMHGI NYHIPLPEQP GGSSDFIAFT  
 551 DKAKLTFTTS RPMKMVYITD ETEPSLTSTP YTVPLEFAQT GLLKIRTVTA  
 601 GKGMSPVVRI RVEKQPFNMS MEVPAPKPGI TIRTAYGDLY DVPDLQQVAS  
 651 WEVGTVSSLE EIMHGKEKIT SPEVLERRV EATGYVLIPE DGVYEFSTEN  
 701 NEFWIDNVKL IDNVGEVKKF SRRNSSRALQ KGYHPIKTIW VGAIQGAWPT  
 751 YWNYSRVMIR LKGEKFKPI SSDMLFQ

## 2) INFORMATION FOR SEQ ID NO: 4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 562 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

1 MVLDMKIIFH LLLWLNCNVV HAAKVEILPA PQSVTWENDT AIIINPRLLQ  
 51 ANTSCPLLED AFVRTVSAIE KLKWHPPPID DFNTANGKNI KTSLVHIQVD  
 101 DATVDLQLGV NESYTLKINT DGINIHAATT WGALHGLVSL QQLIHTSED  
 151 KYVVPISVTI SDFPNFKHRG LMIDSGRNFL TVDSILEQID IMALSKMNSL  
 201 HWHLADSQSW PVALESYPHM IKDAYSNDV YSKNDLKYIV DYARARGVRV  
 251 IPEIDMPGHA RAGWKQVDPT IVECADAFWT DAAVEPPPQ LNISEKTYE  
 301 VISNVYNELS DIFIDVPEHV GNDELQEKCY SAQLLPNNTV TDLLKRYLKK  
 351 ALPIFNKVNH RKLTMWDDVL LSDVSADKIP SNITLQVWHE ISGVKNLTSR  
 401 GYDVVVSLSL FLYLDCGNAG WVTNDPRYVE TPENVDFNTG QGGSWCGPYK  
 451 SYQRIYNFDF TANLTETERN HVLGREAAW SEQVDSTVLT TKIWPRTAAL  
 501 AELTWSGNKD SNGHHRGYEF TQRILNFREY LVKLGYGVSP LVPKYCLLNP  
 551 HACDLYKNPP VY

## 2) INFORMATION FOR SEQ ID NO: 5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 847 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

1 MASDIDQKDV DYAAKNLKL TSLVANKPKD CPPEAPWGAC YRVEINLENT  
 51 GSKSLNENVE IYFSSIHR TL GSKSEEFKVE HINGDLHKIT TTEKPKGLKG  
 101 GRTKSFQVDF MNWIVSNSDF MPNYIVASEH LEGRNINLTV PIDAVHITEE  
 151 VSGFTTGIRK TPNQLKRTAN DLLPAATATT RYBQYSKVKD LGADAVSAHI  
 201 LPTPLETSVH EGSLNIAQGI NIVSDALPAD QVEALNFRFE TLGVNTGTGV  
 251 PVNVTIKADS SKKSGSYTLD VTSSGIRIVG VDKAGAFYGV QSLAGLVTVG  
 301 KDTINQVSIN DEPRLDYRGM HMDVSRNFHS KELVFRFLDQ MAAYKMNKPH  
 351 FHLADDEGWR LEINGLPELT QVGAHRCHDV EQNKCMMPQL GSGAELPNNG  
 401 SGYYTREDYK EILAYASARN IQVIPSMDMP GHSLAAVKSM EARYRKFMAR  
 451 GDVVKAEMYL LSDPNDTTQY YSIQHYQDNT INPCMESSFV FMDKVIDEIN  
 501 KLHKEGGQPL TDYHIGADET AGAWGDSPEC RKMFVAPESG VKNKADINGY  
 551 FINRISHILD AKGLTLGAWN DGLSHKALDA SSLAGNPPKA WVGTMFWGG  
 601 VDQYNSFANK GYDVVVTPPD AYYFDMPIYEN DPEERGGYWA TRFNDTKKVF  
 651 SPMPENVPAN VEWMTDRMGA KISATTGEKT HDPLGVQGAL WSETIRTDQA  
 701 VEYMLPRMI AVAERGWHKA SWEKEHKEGI TYTSNVDGHE GTTHLNDNIA  
 751 TRDADWAHFS NILGYKEMPK LDKAGITYRL PVLGAVIKNN ILDVVTEFHG  
 801 VAIQYSLD GK TWKHYDDTKK PQVSTKALVR SVSTNGRTGR AVEVLAK

## 2) INFORMATION FOR SEQ ID NO: 6:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1589 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

1 atgacaagct ccaggctttg gtttctgctg ctgctggcgg cagcgttcgc  
 51 aggaacggggc acggccctct gccctggcc tcagaacttc caaacctccg  
 101 accagcgcta cgtcctttac ccgaacaact ttcaattcca gtacgatgac  
 151 agctcggccg cgcagcccg ctgctcagtc ctgacgagg ccttcacgcg  
 201 ctatcgtgac ctgcttttcg gttccgggtc ttggcccggt ccttacctca  
 251 cagggaaacg gcatacactg gagaagaatg tgttggttgt ctctgtatgc  
 301 acacctggat gtaaccagct tcctactttg gagtcagtgg agaattatac  
 351 cctgaccata aatgatgacc agtgtttact cctctctgag actgtctggg  
 401 gagctctccg aggtctggag acttttagcc agcttggttg gaaatctgct  
 451 gagggacat tctttatcaa caagactgag attgaggact tccccgctt  
 501 tctcaccgg ggcttgctgt tggatacatc tcgccattac ctgccactct  
 551 ctagcatcct ggacactctg gatgtcatgg cgtacaataa attgaacgtg  
 601 ttccactggc atctggtaga tgatccttcc tcccatatg agagcttcac

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651 ttttccagag ctcatgagaa aggggtccta caaccctgtc acccacatct  
 701 acacagcaca ggatgtgaag gaggtcattg aatacgcacg gctccggggg  
 751 atccgtgtgc ttgcagagtt tgacactcct ggccacactt tgccttgggg  
 801 accaggtatc cctggattac tgactccttg ctactctggg tctgagccct  
 851 ctggcacctt tggaccagtg aatcccagtc tcaataatac ctatgagttc  
 901 atgagcacat tcttcttaga agtcagctct gtcttcccag attttatctt  
 951 catcttgagg gagatgaggt tgatttcacc tgctggaagt ccaaccaga  
 1001 gatccaggac tttatgagga agaaaggctt cggtgaggac ttcaagcagc  
 1051 tggagtcctt ctacatccag acgtgtctgg acatcgtctc ttcttatggc  
 1101 aagggtctatg tgggtgtggca ggaggtgttt gataataaag taaagattca  
 1151 gccagacaca atcatacagg tgtggcgaga ggatattcca gtgaactata  
 1201 tgaaggagct ggaactggtc accaaggccg gcttccgggc ccttctctct  
 1251 gccccctggg acctgaaccg tatatcctat ggccctgact ggaaggattt  
 1301 ctacgtagtg gaacccttg catttgaagg taccctgag cagaaggctc  
 1351 tgggtgattgg tggagaggct tgtatgtggg gagaatatgt ggacaacaca  
 1401 aacctggtcc ccaggctctg gccagagca ggggctgttg ccgaaaggct  
 1451 gtggagcaac aagttgacat ctgacctgac atttgcctat gaacgtttgt  
 1501 cacacttccg ctgtgagttg ctgaggcgag gtgtccaggc ccaaccctc  
 1551 aatgtaggct tctgtgagca ggagtttgaa cagacctga

## 2) INFORMATION FOR SEQ ID NO: 7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3670 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (iii) HYPOTHETICAL: NO

## (iii) ANTI-SENSE: NO

## (v) FRAGMENT TYPE: internal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

1 ggtgggtggca cctcctgcg cgcggtattc ggcatgcgtc cggcgtttga  
 51 ttggcgacag gaccggcagc gccaacctgt tgcttggcgt ggaacgcgat  
 101 ggacgcgctc attcacgcca tcaccttagc tgccgaacaa ggccggcctga  
 151 ataacgataa ctttgggtcaa ctgcacgtgg gcttggcgct ggctggcggtg  
 201 agcaccaagc gacttggcat gctttatgca attgccacac cgtttgcgtc  
 251 gctcacgctc aataccgatg cctatgggtc gtgcctcggt gcgcaccaag  
 301 gtgacaacgg cgccatcatg attgctggca cgggctcatg cggtttgttc  
 351 ttgcaagacg gccaccagca cgtggtgggg ggacgtgagt tcccgatctc  
 401 cgatgagggc agtggcgcgg tgatgggact gcgcctgatt caacaagtgc  
 451 tgctgattga agatgggtatt tatccggcca cgccacttag tcagtgtgtc  
 501 atgcagcatt gacacgatgt gacgccattg tcgcttggtc gaaatccgct

551 ttacctcgcg actatgggtca attttcgccg cagattttcg cgttggcgaa  
601 tcaaggtgac acgctagcaa tatccctgct gaaacagaca gcagcggata  
651 tcgaaatgtt tttgaacgcc ctgcacgcga aaggggcaca gcgaatctgc  
701 ttcattggga gcacgcgga acgcattcac gcattggtat cccctcccg  
751 tcagcaatgg atcgtcgcac cgcaagcggg tgcgatggag ggcgcattaa  
801 tgtttgcccg caaagccgag cataatttgt attaaggggt gctcatgaac  
851 tatcgaatag acttcgcggt attgtcagaa catccacagt tctgcggtt  
901 tggcttgacg ctgcataacc tcagcgatca ggacttaaag gcctggagcc  
951 tgcatttcac catcgatcgc tacattcagc ccgatagcat cagtcacagc  
1001 cagattcatc aagtcggcag tttctgttcg ctacgcggg agcaggacgt  
1051 gataaattcc aacagccatt tctactgca attcagcatc aaaaccgcgc  
1101 cgtttccggt tcaactattac accgacggca tcaaagccgc gtttgtccaa  
1151 attaatgatg tagagccgcg ggttcgctac gacgtgatcg tcacccccat  
1201 cgcactcgcc tccccctac gggaacgcag cgagatcccg gccacggatg  
1251 ccgcgacgtt gacgctgta cccaaacca atcatatcga acgcttggat  
1301 ggtgaatttg cccttacgc cggcagccag atttcattgc aatcctcttg  
1351 tgcagaaact gccgccacgt ggctcaagca agaactgacg catctctatc  
1401 agtggcagcc acacgatatt ggcagcgccg acattgtgct acgcaccaac  
1451 ccaacgctgg atgaaggcgc ctatctgctg tcagtcgacc gcaaacctat  
1501 tcgtttggaa gccagcagtc acatcggctt tgtccatgcc agtgcgacat  
1551 tgctgcaatt ggttcgcccga gatggcgaca acctgctggt gccacacatc  
1601 gttatcaaaag acgcacgcgc ctttaaatac cgcggcatga tgctggattg  
1651 cgcgcgtcat tttcatccgc tggagcgcgt taaacgcctc atcaaccaac  
1701 tggcgcatta caaattcaac acctttcatt ggcatctgac cgatgatgaa  
1751 ggttggcgca ttgaaattaa gtctctacct caattgaccg acattggcgc  
1801 gtggcgcggt gtggatgaag tcctggaacc gcaatacagc ctgctgaccg  
1851 aaaaacacgg tggcttttac acccaagagg agatccgtga agtgcgccc  
1901 tacgcgcgag aacgcggcat cacggtgatt ccagaaattg acattcccgg  
1951 tcacagccga gcggcgatca aagccttacc ggaatggcta tttgacgaag  
2001 atgaccaatc acaataccgc agcatcagc actacaacga caacgtgcta  
2051 tcgccagccc tgcccggcac ctaccgtttt ctcgattgcy tattggagga  
2101 agtggccgcy ctgtttccga gccatttcat tcacattggc gccgatgaag  
2151 tgccagatgg cgtgtgggtc aacagccgca aatgtcaggc attgatggca  
2201 gaagagggct acaccgacgc caaagagtta caagggcacc tgctgcgcta  
2251 tgccggagaag aagctcaaact cactcggcaa acgcattggt ggttgggaag  
2301 aagcgcagca tggtgacaaa gtcagcaaaag ataccgtgat ttattcttgg  
2351 ttatcogaac aagcgcact gaactgcgcc cgtcaagggg ttgatgtcat  
2401 tttacaaccg ggacagttaa cgtacctcga cattgcgcaa gactacgcgc  
2451 cagaagagcc gggcgtcgac tgggctggcg tgacgccact ggagcgcgcc  
2501 tatcgctacg agccgctggg cgaggtgcca gaacacgacc cgtgcgcaa  
2551 acgcattttg gggattcagt gcgcgctgtg gtgtgaactg gtcaacaatc  
2601 aagaccgcat ggactacatg atctatccgc gtttgaccgc actggcggga

2651 agcggcttgg acacaaaaat cccagcgtga ttggctggat tacctggcgc  
 2701 gcctcaaagg ccatttacct caacttgatc aacaaggcat ccgtaccgg  
 2751 gcgccttggg aagcataacg caacacgttt tctctagcat cgacattgag  
 2801 tggcgccaat gcgccactgt ttaaaaagga aattaccatg aaatacggct  
 2851 atttcgataa cgacaatcgc gaatacgtca ttactcgtcc cgatgttcct  
 2901 gcaccttggg ccaactacct cggcacggaa aaattctgca ccgtcatctc  
 2951 ccataatgcy gggggctact cgttctatca ctcacccgag tacaaccgtg  
 3001 tgaccaagtt ccgtccgaac ttcacacaag atcgtcccgg gcattacatc  
 3051 tatttgcgcy atgatgaaac cgggtgatttc tggtcgggtct cttggcagcc  
 3101 cgttgccaaa aaccttgacy atgcccatta cgaagtgcgc catggatgcc  
 3151 gtgtatgagt atctgttctc cccatacggg ttacacctca acgccccctc  
 3201 gtttgcaacy cccaacgatg acatcgggtt tgtcaccgcg gtctaccaag  
 3251 gcgtgaaaga aaacgggtgc attttctcgc atccgaacct gtgggcattg  
 3301 gtgcgcgaag ccaaactggg acgcgggtgat cgcgcgatgg aattctacga  
 3351 ttcgctcaac ccatacaacc agaacgacat cattgaaacy cgcgtggcag  
 3401 agccatattc ctacgtgcaa ttcacatggt gtcgcgacca ccaagatcac  
 3451 ggccgtgcaa accacccttg gtcacccggg acatcggggt gggcctacta  
 3501 cgcgaccacc aacttcattt tgggagtgcg taccggattt gacaggttga  
 3551 ccgtggatcc atgtattcct gccgcttggg cgggctttga gcgtcacgcg  
 3601 cgagtggcgc ggtgcgacgt atcacatgct agtccaaaac ccgaatggcg  
 3651 tcagcaaagg cgtgcaatcg

2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2000 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (B) STRAIN: *Trichoderma harzianum* CBS 243.71

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 86..1819

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GACATCTCCA CCATAGAGTC GACTCATTGC TGGCATACGG AGCATTCCAA TCTTACTCGT  
60

AGTAGTGTTA TTGCCATCGC TCATC ATG CTG CCC AAG GCG ATC ATC GCG ATT  
112

Met Leu Pro Lys Ala Ile Ile Ala Ile

1

5

GCC GCA TTG GCT TTC AGC CCA GCA AAT GCG CTG TGG CCC ATT CCT CAG  
160

45

Ala Ala Leu Ala Phe Ser Pro Ala Asn Ala Leu Trp Pro Ile Pro Gln  
 10 15 20 25

AAG ATC TCG ACC GGA GAC AGC GTG CTC TTT ATT GAC CAG GCT GTT AGG  
 208

Lys Ile Ser Thr Gly Asp Ser Val Leu Phe Ile Asp Gln Ala Val Arg  
 30 35 40

GTG ACT TAC AAT GGA GTA CCG ATC ATC CCT ATC GGC TAC AAC CCA CCG  
 256

Val Thr Tyr Asn Gly Val Pro Ile Ile Pro Ile Gly Tyr Asn Pro Pro  
 45 50 55

GCC AGC TCC AAC TTC GAC AGC AGG CAA ATC GTC CAG GCG GCT GTC TCG  
 304

Ala Ser Ser Asn Phe Asp Ser Arg Gln Ile Val Gln Ala Ala Val Ser  
 60 65 70

CGC GCT TTC CAA AAC ATC TTC AGC ACC AAC TAT GTG CCA TGG AAG CTT  
 352

Arg Ala Phe Gln Asn Ile Phe Ser Thr Asn Tyr Val Pro Trp Lys Leu  
 75 80 85

CAC CCG CGT AAC AGC AAC TTT GAG CCG AAG GTG GCC CCT CAG AAC CGA  
 400

His Pro Arg Asn Ser Asn Phe Glu Pro Lys Val Ala Pro Gln Asn Arg  
 90 95 100 105

ATC CAG TCC ATC TCA ATT CAG CAG ACT GGA AAG GAT ACG TCC AAG ACG  
 448

Ile Gln Ser Ile Ser Ile Gln Gln Thr Gly Lys Asp Thr Ser Lys Thr  
 110 115 120

TTC AAG CCG CGC GCC GGA GAC GTT GAT GAG TCG TAC TCT TTG ACC ATT  
 496

Phe Lys Pro Arg Ala Gly Asp Val Asp Glu Ser Tyr Ser Leu Thr Ile  
 125 130 135

TCC AAG AAT GGA CAG GTC AAC ATC AGT GCC AAG TCT TCT ACT GGT GTG  
 544

Ser Lys Asn Gly Gln Val Asn Ile Ser Ala Lys Ser Ser Thr Gly Val  
 140 145 150

CTG CAC GCC CTC GAG ACC TTC TCG CAG CTT TTC TAC AAG CAC TCT GCT  
592

Leu His Ala Leu Glu Thr Phe Ser Gln Leu Phe Tyr Lys His Ser Ala  
155 160 165

GGA CCT TTC TAC TAT ACG ACT CAG GCT CCC GTG TCC ATC ACA GAC GCT  
640

Gly Pro Phe Tyr Tyr Thr Thr Gln Ala Pro Val Ser Ile Thr Asp Ala  
170 175 180 185

CCC AAA TAT CCC CAC CGT GGC ATC ATG CTT GAC CTT GCC CGT AAC TAT  
688

Pro Lys Tyr Pro His Arg Gly Ile Met Leu Asp Leu Ala Arg Asn Tyr  
190 195 200

CAA ACC ATT GAT GAC ATC AAG AGG ACC ATT GAC GCC ATG TCG TGG AAC  
736

Gln Thr Ile Asp Asp Ile Lys Arg Thr Ile Asp Ala Met Ser Trp Asn  
205 210 215

AAG CTT AAC CGC CTG CAC TTG CAC ATC ACC GAC TCT CAG TCG TGG CCG  
784

Lys Leu Asn Arg Leu His Leu His Ile Thr Asp Ser Gln Ser Trp Pro  
220 225 230

CTG GTG ATC CCC TCG CTG CCT AAG CTG TCC CAG GCC GGT GCC TAC CAC  
832

Leu Val Ile Pro Ser Leu Pro Lys Leu Ser Gln Ala Gly Ala Tyr His  
235 240 245

CCC AGC CTC GTC TAC ACT CCC GCA GAC CTT GCT GGC ATT TTC CAG TAC  
880

Pro Ser Leu Val Tyr Thr Pro Ala Asp Leu Ala Gly Ile Phe Gln Tyr  
250 255 260 265

GGT GTC GCC CGC GGT GTT GAG GTC ATT ACG GAG ATC GAT ATG CCT GGC  
928

Gly Val Ala Arg Gly Val Glu Val Ile Thr Glu Ile Asp Met Pro Gly  
270 275 280

CAC ATC GGT GTT ATC GAG CTC GCT TAC AGC GAT CTC ATT GTT GCC TAC  
976



47

His Ile Gly Val Ile Glu Leu Ala Tyr Ser Asp Leu Ile Val Ala Tyr  
 285 290 295

GAA GAG ATG CCT TAC CAG TAC TAC TGC GCC GAG CCA CCT TGC GGT GCC  
 1024

Glu Glu Met Pro Tyr Gln Tyr Tyr Cys Ala Glu Pro Pro Cys Gly Ala  
 300 305 310

TTT TCC ATC AAC AAC ACC AAG GTG TAC AGC TTC CTC GAT ACC CTG TTC  
 1072

Phe Ser Ile Asn Asn Thr Lys Val Tyr Ser Phe Leu Asp Thr Leu Phe  
 315 320 325

GAC GAC CTT TTG CCT CGC GTC GCT CCT TAC AGC GCG TAC TTC CAC ACC  
 1120

Asp Asp Leu Leu Pro Arg Val Ala Pro Tyr Ser Ala Tyr Phe His Thr  
 330 335 340 345

GGT GGT GAC GAG CTC AAC GCT AAC GAC TCC ATG CTC GAC TCT CAC ATC  
 1168

Gly Gly Asp Glu Leu Asn Ala Asn Asp Ser Met Leu Asp Ser His Ile  
 350 355 360

AAG AGC AAC GAG ACC TCC GTT CTG CAA CCT CTG CTG CAA AAG TTC ATC  
 1216

Lys Ser Asn Glu Thr Ser Val Leu Gln Pro Leu Leu Gln Lys Phe Ile  
 365 370 375

AAC TTT GCC CAC TCC AAG GTC CGT GCC GCG GGC TTG TCG CCA TTT GTC  
 1264

Asn Phe Ala His Ser Lys Val Arg Ala Ala Gly Leu Ser Pro Phe Val  
 380 385 390

TGG GAG GAG ATG GTC ACC ACC TGG AAC CTG ACC CTC GGC AGC GAC ACC  
 1312

Trp Glu Glu Met Val Thr Thr Trp Asn Leu Thr Leu Gly Ser Asp Thr  
 395 400 405

GTC GTT CAG TCG TGG CTG GGT GGC GAT GCC GTC AAG AAC CTG GCT GAG  
 1360

Val Val Gln Ser Trp Leu Gly Gly Asp Ala Val Lys Asn Leu Ala Glu  
 410 415 420 425

AGC GGC CAC AAG GTC ATT GAC ACC GAC TAC AAC TTC TAC TAC TTG GAC  
1408

Ser Gly His Lys Val Ile Asp Thr Asp Tyr Asn Phe Tyr Tyr Leu Asp  
430 435 440

TGC GGC CGT GGT CAA TGG GTC AAC TTC CCT CCA GGA GAC TCC TAC AAC  
1456

Cys Gly Arg Gly Gln Trp Val Asn Phe Pro Pro Gly Asp Ser Tyr Asn  
445 450 455

ACC TAC TAC CCA TTC AAC GAC TGG TGC CAG CCC ACC AAG AAC TGG AGG  
1504

Thr Tyr Tyr Pro Phe Asn Asp Trp Cys Gln Pro Thr Lys Asn Trp Arg  
460 465 470

CTC ATC TAC TCT CAC GAC CCT GCA GCC AAC GTG TCT GCT TCG GCT GCC  
1552

Leu Ile Tyr Ser His Asp Pro Ala Ala Asn Val Ser Ala Ser Ala Ala  
475 480 485

AAG AAC GTC CTG GGA GGA GAG CTT GCC ATT TGG AGC GAG ATG ATT GAC  
1600

Lys Asn Val Leu Gly Gly Glu Leu Ala Ile Trp Ser Glu Met Ile Asp  
490 495 500 505

GCC AGC AAC CTG GAC AAC ATC ATC TGG CCT CGT GGC AGC GCC GCC GGT  
1648

Ala Ser Asn Leu Asp Asn Ile Ile Trp Pro Arg Gly Ser Ala Ala Gly  
510 515 520

GAG GTT TGG TGG TCC GGC AAT ACC GAT GCC TCT GGT GAG CAG CGC AGC  
1696

Glu Val Trp Trp Ser Gly Asn Thr Asp Ala Ser Gly Glu Gln Arg Ser  
525 530 535

CAG CTG GAC GTT GTT CCT CGT CTG AAC GAG TTC CGA GAA CGC TTG CTT  
1744

Gln Leu Asp Val Val Pro Arg Leu Asn Glu Phe Arg Glu Arg Leu Leu  
540 545 550

GCT CGT GGT GTC AGC GCG TTC CCC ATC CAG ATG ACC TAC TGC ACT CAG  
1792

49

Ala Arg Gly Val Ser Ala Phe Pro Ile Gln Met Thr Tyr Cys Thr Gln  
 555 560 565

CTC AAC GCC ACT GCC TGC ACA CTA TTT TAAGTCTAAG ATGACTTTTT  
 1839

Leu Asn Ala Thr Ala Cys Thr Leu Phe  
 570 575

CTTTTATTGG GCAGGGTTTT TTCTATTTTT CACGTATTAT CATTAGTGTA CAGTGATTAA  
 1899

AACAGGTATG GCTTAAGAGG AGCTGGGAGG GTATCCGGCT TGGGGCGGTA TATTATTAAC  
 1959

TGTATATAAT TCAAATTCAT CTACATATAT GTTATGAAAA A  
 2000

## 2) INFORMATION FOR SEQ ID NO: 9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2239 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (vi) ORIGINAL SOURCE:

- (B) STRAIN: *Trichoderma harzianum* CBS 243.71

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 282..2086

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

CTGAGAAGCG GCACTTGCTG ATCTGCGTGG AACTTGGGGT TACAACGCAC CGGATAGCTC  
 60  
 ATCTCCCCAG GACCCCGGAA CTGGAGCTGG AACTGGAATT GGAGCTGGAG CGGACCCAGG  
 120  
 CCGGAGACGA GAAACACAGT GAATCACTCC TGCAAGGGGC GGGACAGGAA CGTGGACAGT  
 180  
 ATTTAGTTTA AGCAGCTGTC CCAGAGCTGT TCGCCCTGCT TCCAAGCTCG TGTGGCCTGA  
 240  
 CCGTGTATAA ACTCATTACG ACCATCAGCT CACAGCCGAC A ATG TTT TCC AGG  
 293

Met Phe Ser Arg

50

GCC ATT GTC GCC GCA TTG GCC CTG AGC GGC CCG GCT TTT GCC CTG TGG  
341

Ala Ile Val Ala Ala Leu Ala Leu Ser Gly Pro Ala Phe Ala Leu Trp  
5 10 15 20

CCC GTG CCT AAA CAC TCC TCG ACC GGC AAT GAC ACG CTC TTT ATT GAC  
389

Pro Val Pro Lys His Ser Ser Thr Gly Asn Asp Thr Leu Phe Ile Asp  
25 30 35

CAG ACG GTC CAG GTT ACC TAC AAT GGT GAA CAG GTG TGG TGG ACT CCT  
437

Gln Thr Val Gln Val Thr Tyr Asn Gly Glu Gln Val Trp Trp Thr Pro  
40 45 50

CCA TAT GAT GAC CCC GGA TCC CCG GAC TTT GCT GAG ACC AGG ATC GAT  
485

Pro Tyr Asp Asp Pro Gly Ser Pro Asp Phe Ala Glu Thr Arg Ile Asp  
55 60 65

GAC CAA CAG GTT ACT TAC ACG GCC GGC TAC GTG CCT CCC AGC GGA CCG  
533

Asp Gln Gln Val Thr Tyr Thr Ala Gly Tyr Val Pro Pro Ser Gly Pro  
70 75 80

CAT TTC ACC AGC AAG GAA ATC GTT CAA GGC GGC GTC TCG CGG ACA TTC  
581

His Phe Thr Ser Lys Glu Ile Val Gln Gly Gly Val Ser Arg Thr Phe  
85 90 95 100

GGC GCC ATC TTC CAG CAG GGC TTT GTG CCG TGG ATG CTG CGT GAA CGT  
629

Gly Ala Ile Phe Gln Gln Gly Phe Val Pro Trp Met Leu Arg Glu Arg  
105 110 115

GAT TCG AAC TCT GAA CCG AAT CTA GGC GGA ACG CGG ATC CGG ACA CTG  
677

Asp Ser Asn Ser Glu Pro Asn Leu Gly Gly Thr Arg Ile Arg Thr Leu  
120 125 130

CAG ATT ATA CAG ACT CAG CAC GAT TCT GCG AAT ACC TTC AAG CCT CTG  
725

51

Gln Ile Ile Gln Thr Gln His Asp Ser Ala Asn Thr Phe Lys Pro Leu  
 135 140 145

AAT GGC GCA GTG AAT GAA TCC TAT GCC CTG GAT GTC GAC GCA AAG GGC  
 773

Asn Gly Ala Val Asn Glu Ser Tyr Ala Leu Asp Val Asp Ala Lys Gly  
 150 155 160

CAC GCA TCT CTC ACC GCT CCG TCG TCA ACG GGC ATC CTT CGA GGC CTT  
 821

His Ala Ser Leu Thr Ala Pro Ser Ser Thr Gly Ile Leu Arg Gly Leu  
 165 170 175 180

GAG ACC TTC TCC CAG CTC TTC TTC AAG CAT AGC TCC GGC ACT GCT TGG  
 869

Glu Thr Phe Ser Gln Leu Phe Phe Lys His Ser Ser Gly Thr Ala Trp  
 185 190 195

TAT ACG CAG CTT GCA CCT GTT TCG ATC CGC GAT GAG CCC AAG TAT CCT  
 917

Tyr Thr Gln Leu Ala Pro Val Ser Ile Arg Asp Glu Pro Lys Tyr Pro  
 200 205 210

CAC CGC GGC CTC CTG TTG GAT GTC AGC CGC CAT TGG TTC GAG GTT TCC  
 965

His Arg Gly Leu Leu Leu Asp Val Ser Arg His Trp Phe Glu Val Ser  
 215 220 225

GAC ATT GAG CGC ACT ATC GAT GCT CTG GCC ATG AAC AAA ATG AAT GTG  
 1013

Asp Ile Glu Arg Thr Ile Asp Ala Leu Ala Met Asn Lys Met Asn Val  
 230 235 240

CTG CAT CTG CAC GCT ACT GAC ACG CAG TCA TGG CCG CTG GAG ATT CCA  
 1061

Leu His Leu His Ala Thr Asp Thr Gln Ser Trp Pro Leu Glu Ile Pro  
 245 250 255 260

TCC CTG CCT CTG CTG GCT GAG AAG GGC GCC TAT CAC AAG GGT TTG AGC  
 1109

Ser Leu Pro Leu Leu Ala Glu Lys Gly Ala Tyr His Lys Gly Leu Ser  
 265 270 275

TAC TCG CCA AGC GAT CTT GCG AGC ATC CAA GAA TAT GGT GTT CAT CGA  
1157

Tyr Ser Pro Ser Asp Leu Ala Ser Ile Gln Glu Tyr Gly Val His Arg  
280 285 290

GGT GTC CAG GTC ATT GTA GAG ATT GAT ATG CCG GGC CAC GTT GGA ATC  
1205

Gly Val Gln Val Ile Val Glu Ile Asp Met Pro Gly His Val Gly Ile  
295 300 305

GAC AAG GCA TAC CCC GGG CTT AGC AAC GCC TAC GGA GTC AAC CCG TGG  
1253

Asp Lys Ala Tyr Pro Gly Leu Ser Asn Ala Tyr Gly Val Asn Pro Trp  
310 315 320

CAG TGG TAC TGC GCC CAG CCG CCC TGC GGA TCT TTC AAG CTG AAC AAC  
1301

Gln Trp Tyr Cys Ala Gln Pro Pro Cys Gly Ser Phe Lys Leu Asn Asn  
325 330 335 340

ACG GAT GTC GAA AAG TTC ATT GAC AAG CTG TTT GAA GAT TTG CTG CCG  
1349

Thr Asp Val Glu Lys Phe Ile Asp Lys Leu Phe Glu Asp Leu Leu Pro  
345 350 355

CGT CTT TCG CCG TAC TCG GCC TAC TTT CAC ACT GGT GGC GAT GAG TAC  
1397

Arg Leu Ser Pro Tyr Ser Ala Tyr Phe His Thr Gly Gly Asp Glu Tyr  
360 365 370

AAG GCG AAC AAC TCG CTG CTC GAC CCG GCC CTT CGC ACA AAC GAC ATG  
1445

Lys Ala Asn Asn Ser Leu Leu Asp Pro Ala Leu Arg Thr Asn Asp Met  
375 380 385

AAC ACC CTG CAG CCG ATG CTG CAG CGC TTC TTG GAC CAC GTG CAT GGC  
1493

Asn Thr Leu Gln Pro Met Leu Gln Arg Phe Leu Asp His Val His Gly  
390 395 400

AAA GTT CGT GAT CTG GGA CTC GTT CCC ATG GTT TGG GAA GAA ATG ATT  
1541

53

Lys Val Arg Asp Leu Gly Leu Val Pro Met Val Trp Glu Glu Met Ile  
 405 410 415 420

CTG GAT TGG AAC GCA ACT CTG GGC AAG GAT GTC GTT GCT CAA ACG TGG  
 1589

Leu Asp Trp Asn Ala Thr Leu Gly Lys Asp Val Val Ala Gln Thr Trp  
 425 430 435

CTT GGC GGA GGA GCG ATT CAG AAG CTT GCT CAG GCT GGA TAC AAG GTT  
 1637

Leu Gly Gly Gly Ala Ile Gln Lys Leu Ala Gln Ala Gly Tyr Lys Val  
 440 445 450

ATT GAC AGC AGC AAT GAC TTT TAC TAT CTC GAC TGT GGT CGT GGT GAG  
 1685

Ile Asp Ser Ser Asn Asp Phe Tyr Tyr Leu Asp Cys Gly Arg Gly Glu  
 455 460 465

TGG CTC GAT TTT GCC AAT GGT GAC CCC TTT AAC AAC AAC TAT CCC TTT  
 1733

Trp Leu Asp Phe Ala Asn Gly Asp Pro Phe Asn Asn Asn Tyr Pro Phe  
 470 475 480

CTC GAC TGG TGC GAC CCG ACC AAA AAC TGG AAG CTC ATG TAC TCA CAC  
 1781

Leu Asp Trp Cys Asp Pro Thr Lys Asn Trp Lys Leu Met Tyr Ser His  
 485 490 495 500

GAG CCC ACG GAC GGC GTG TCC GAT GAT CTC AAG AAG AAT GTC ATT GGA  
 1829

Glu Pro Thr Asp Gly Val Ser Asp Asp Leu Lys Lys Asn Val Ile Gly  
 505 510 515

GGC GAA GTT GCT GTC TGG ACT GAG ACC ATC GAT CCG ACC AGC TTG GAC  
 1877

Gly Glu Val Ala Val Trp Thr Glu Thr Ile Asp Pro Thr Ser Leu Asp  
 520 525 530

TCC ATC ATC TGG CCG CGA GCG GGA GCG GCC GCT GAG ATT TGG TGG TCG  
 1925

Ser Ile Ile Trp Pro Arg Ala Gly Ala Ala Ala Glu Ile Trp Trp Ser  
 535 540 545

GGC AAG ATC GAT GAG AAG GGC CAG AAC CGA TCA CAG ATT GAT GCA CGG  
1973

Gly Lys Ile Asp Glu Lys Gly Gln Asn Arg Ser Gln Ile Asp Ala Arg  
550 555 560

CCA AGA TTA TCG GAG CAG CGA GAG CGC ATG TTG GCG AGG GGA GTT CGA  
2021

Pro Arg Leu Ser Glu Gln Arg Glu Arg Met Leu Ala Arg Gly Val Arg  
565 570 575 580

GGA ACG CCG ATT ACG CAG CTG TGG TGT AGT CAG GTT GAT GTT CAT AAC  
2069

Gly Thr Pro Ile Thr Gln Leu Trp Cys Ser Gln Val Asp Val His Asn  
585 590 595

TGC GAG TCT GGG AAT TA CTGATGCGGG TTGATGAACA AAGTATGTAA  
2116

Cys Glu Ser Gly Asn  
600

TGTGGTATAT ATGAATGTTT CTTTTTCACG CTGCTGTAA AGGCCGGGGA CGTCTCGTT  
2176

GTGATGACGG TTAGACTGAA AATCACTTAT AATGAATTCA AGTCATTCAA GATGAAAAAA  
2236

AAA  
2239

2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 578 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Met Leu Pro Lys Ala Ile Ile Ala Ile Ala Ala Leu Ala Phe Ser Pro  
1 5 10 15  
Ala Asn Ala Leu Trp Pro Ile Pro Gln Lys Ile Ser Thr Gly Asp Ser  
20 25 30



Val Leu Phe Ile Asp Gln Ala Val Arg Val Thr Tyr Asn Gly Val Pro  
 35 40 45

Ile Ile Pro Ile Gly Tyr Asn Pro Pro Ala Ser Ser Asn Phe Asp Ser  
 50 55 60

Arg Gln Ile Val Gln Ala Ala Val Ser Arg Ala Phe Gln Asn Ile Phe  
 65 70 75 80

Ser Thr Asn Tyr Val Pro Trp Lys Leu His Pro Arg Asn Ser Asn Phe  
 85 90 95

Glu Pro Lys Val Ala Pro Gln Asn Arg Ile Gln Ser Ile Ser Ile Gln  
 100 105 110

Gln Thr Gly Lys Asp Thr Ser Lys Thr Phe Lys Pro Arg Ala Gly Asp  
 115 120 125

Val Asp Glu Ser Tyr Ser Leu Thr Ile Ser Lys Asn Gly Gln Val Asn  
 130 135 140

Ile Ser Ala Lys Ser Ser Thr Gly Val Leu His Ala Leu Glu Thr Phe  
 145 150 155 160

Ser Gln Leu Phe Tyr Lys His Ser Ala Gly Pro Phe Tyr Tyr Thr Thr  
 165 170 175

Gln Ala Pro Val Ser Ile Thr Asp Ala Pro Lys Tyr Pro His Arg Gly  
 180 185 190

Ile Met Leu Asp Leu Ala Arg Asn Tyr Gln Thr Ile Asp Asp Ile Lys  
 195 200 205

Arg Thr Ile Asp Ala Met Ser Trp As Lys Leu Asn Arg Leu His Leu  
 210 215 220

His Ile Thr Asp Ser Gln Ser Trp Pro Leu Val Ile Pro Ser Leu Pro  
 225 230 235 240

Lys Leu Ser Gln Ala Gly Ala Tyr His Pro Ser Leu Val Tyr Thr Pro  
 245 250 255

Ala Asp Leu Ala Gly Ile Phe Gln Tyr Gly Val Ala Arg Gly Val Glu  
 260 265 270

Val Ile Thr Glu Ile Asp Met Pro Gly His Ile Gly Val Ile Glu Leu  
 275 280 285

Ala Tyr Ser Asp Leu Ile Val Ala Tyr Glu Glu Met Pro Tyr Gln Tyr  
 290 295 300

Tyr Cys Ala Glu Pro Pro Cys Gly Ala Phe Ser Ile Asn Asn Thr Lys  
 305 310 315 320

Val Tyr Ser Phe Leu Asp Thr Leu Phe Asp Asp Leu Leu Pro Arg Val  
 325 330 335

Ala Pro Tyr Ser Ala Tyr Phe His Thr Gly Gly Asp Glu Leu Asn Ala  
 340 345 350

Asn Asp Ser Met Leu Asp Ser His Ile Lys Ser Asn Glu Thr Ser Val  
 355 360 365

Leu Gln Pro Leu Leu Gln Lys Phe Ile Asn Phe Ala His Ser Lys Val  
 370 375 380

Arg Ala Ala Gly Leu Ser Pro Phe Val Trp Glu Glu Met Val Thr Thr  
 385 390 395 400

Trp Asn Leu Thr Leu Gly Ser Asp Thr Val Val Gln Ser Trp Leu Gly  
 405 410 415

Gly Asp Ala Val Lys Asn Leu Ala Glu Ser Gly His Lys Val Ile Asp  
 420 425 430

Thr Asp Tyr Asn Phe Tyr Tyr Leu Asp Cys Gly Arg Gly Gln Trp Val  
 435 440 445

Asn Phe Pro Pro Gly Asp Ser Tyr Asn Thr Tyr Tyr Pro Phe Asn Asp  
 450 455 460

Trp Cys Gln Pro Thr Lys Asn Trp Arg Leu Ile Tyr Ser His Asp Pro  
 465 470 475 480

57

Ala Ala Asn Val Ser Ala Ser Ala Ala Lys Asn Val Leu Gly Gly Glu  
 485 490 495

Leu Ala Ile Trp Ser Glu Met Ile Asp Ala Ser Asn Leu Asp Asn Ile  
 500 505 510

Ile Trp Pro Arg Gly Ser Ala Ala Gly Glu Val Trp Trp Ser Gly Asn  
 515 520 525

Thr Asp Ala Ser Gly Glu Gln Arg Ser Gln Leu Asp Val Val Pro Arg  
 530 535 540

Leu Asn Glu Phe Arg Glu Arg Leu Leu Ala Arg Gly Val Ser Ala Phe  
 545 550 555 560

Pro Ile Gln Met Thr Tyr Cys Thr Gln Leu Asn Ala Thr Ala Cys Thr  
 565 570 575

Leu Phe

2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 601 base pairs

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Met Phe Ser Arg Ala Ile Val Ala Ala Leu Ala Leu Ser Gly Pro Ala  
 1 5 10 15

Phe Ala Leu Trp Pro Val Pro Lys His Ser Ser Thr Gly Asn Asp Thr  
 20 25 30

Leu Phe Ile Asp Gln Thr Val Gln Val Thr Tyr Asn Gly Glu Gln Val  
 35 40 45

Trp Trp Thr Pro Pro Tyr Asp Asp Pro Gly Ser Pro Asp Phe Ala Glu  
 50 55 60

Thr Arg Ile Asp Asp Gln Gln Val Thr Tyr Thr Ala Gly Tyr Val Pro  
 65 70 75 80

Pro Ser Gly Pro His Phe Thr Ser Lys Glu Ile Val Gln Gly Gly Val  
85 90 95

Ser Arg Thr Phe Gly Ala Ile Phe Gln Gln Gly Phe Val Pro Trp Met  
100 105 110

Leu Arg Glu Arg Asp Ser Asn Ser Glu Pro Asn Leu Gly Gly Thr Arg  
115 120 125

Ile Arg Thr Leu Gln Ile Ile Gln Thr Gln His Asp Ser Ala Asn Thr  
130 135 140

Phe Lys Pro Leu Asn Gly Ala Val Asn Glu Ser Tyr Ala Leu Asp Val  
145 150 155 160

Asp Ala Lys Gly His Ala Ser Leu Thr Ala Pro Ser Ser Thr Gly Ile  
165 170 175

Leu Arg Gly Leu Glu Thr Phe Ser Gln Leu Phe Phe Lys His Ser Ser  
180 185 190

Gly Thr Ala Trp Tyr Thr Gln Leu Ala Pro Val Ser Ile Arg Asp Glu  
195 200 205

Pro Lys Tyr Pro His Arg Gly Leu Leu Leu Asp Val Ser Arg His Trp  
210 215 220

Phe Glu Val Ser Asp Ile Glu Arg Thr Ile Asp Ala Leu Ala Met Asn  
225 230 235 240

Lys Met Asn Val Leu His Leu His Ala Thr Asp Thr Gln Ser Trp Pro  
245 250 255

Leu Glu Ile Pro Ser Leu Pro Leu Leu Ala Glu Lys Gly Ala Tyr His  
260 265 270

Lys Gly Leu Ser Tyr Ser Pro Ser Asp Leu Ala Ser Ile Gln Glu Tyr  
275 280 285

Gly Val His Arg Gly Val Gln Val Ile Val Glu Ile Asp Met Pro Gly  
290 295 300

His Val Gly Ile Asp Lys Ala Tyr Pro Gly Leu Ser Asn Ala Tyr Gly  
305 310 315 320

Val Asn Pro Trp Gln Trp Tyr Cys Ala Gln Pro Pro Cys Gly Ser Phe  
325 330 335

Lys Leu Asn Asn Thr Asp Val Glu Lys Phe Ile Asp Lys Leu Phe Glu  
340 345 350

Asp Leu Leu Pro Arg Leu Ser Pro Tyr Ser Ala Tyr Phe His Thr Gly  
355 360 365

Gly Asp Glu Tyr Lys Ala Asn Asn Ser Leu Leu Asp Pro Ala Leu Arg  
370 375 380

Thr Asn Asp Met Asn Thr Leu Gln Pro Met Leu Gln Arg Phe Leu Asp  
385 390 395 400

His Val His Gly Lys Val Arg Asp Leu Gly Leu Val Pro Met Val Trp  
405 410 415

Glu Glu Met Ile Leu Asp Trp Asn Ala Thr Leu Gly Lys Asp Val Val  
420 425 430

Ala Gln Thr Trp Leu Gly Gly Gly Ala Ile Gln Lys Leu Ala Gln Ala  
435 440 445

Gly Tyr Lys Val Ile Asp Ser Ser Asn Asp Phe Tyr Tyr Leu Asp Cys  
450 455 460

Gly Arg Gly Glu Trp Leu Asp Phe Ala Asn Gly Asp Pro Phe Asn Asn  
465 470 475 480

Asn Tyr Pro Phe Leu Asp Trp Cys Asp Pro Thr Lys Asn Trp Lys Leu  
485 490 495

Met Tyr Ser His Glu Pro Thr Asp Gly Val Ser Asp Asp Leu Lys Lys  
500 505 510

Asn Val Ile Gly Gly Glu Val Ala Val Trp Thr Glu Thr Ile Asp Pro  
515 520 525

60

Thr Ser Leu Asp Ser Ile Ile Trp Pro Arg Ala Gly Ala Ala Ala Glu  
530 535 540

Ile Trp Trp Ser Gly Lys Ile Asp Glu Lys Gly Gln As Arg Ser Gln  
545 550 555 560

Ile Asp Ala Arg Pro Arg Leu Ser Glu Gln Arg Glu Arg Met Leu Ala  
565 570 575

Arg Gly Val Arg Gly Thr Pro Ile Thr Gln Leu Trp Cys Ser Gln Val  
580 585 590

Asp Val His As Cys Glu Ser Gly Asn  
595 600

What is claimed is:

1. A laundry or cleaning product comprising one or more hexosaminidase enzymes.
2. A laundry or cleaning product according to Claim 1 wherein said hexosaminidase enzyme is selected from an enzyme which:
  - i) is encoded by a DNA sequence comprising or included in at least one of the sequences of SEQ ID Nos 6-9, or a sequence homologous thereto encoding a hexosaminidase polypeptide,
  - ii) is immunologically reactive with an antibody raised against a highly purified hexosaminidase encoded by the DNA sequence defined in i), and is specific for hexosaminidase,
  - iii) is immunologically reactive with an antibody raised against a highly purified hexosaminidase having SEQ ID Nos 1-5, 10 or 11, and is specific for hexosaminidase, or
  - iv) is a hexosaminidase having SEQ ID Nos 1-5, 10 or 11, or a hexosaminidase polypeptide sequence homologous thereto.
3. A laundry or cleaning product according to either of Claims 1 or 2 wherein said hexosaminidase enzymes are hexosaminidases having MIC for antimicrobial activity of less than 0.125%, more preferably less than 0.025%, and/or the ability to remove biofilm.
4. A laundry or cleaning product according to any of Claims 1-3 further comprising laundry or cleaning composition ingredients selected from the group consisting of detergent surfactants, detergent enzymes, builders, bleaching agents, and mixtures thereof.
5. A laundry or cleaning product according to any of Claims 1-4 wherein the detergent enzyme is selected from the group consisting of proteases, amylases, lipases, cellulases, and mixtures thereof.
6. A laundry or cleaning product according to any of Claims 1-5 wherein the builder is selected from the group consisting of zeolite, phosphate, and mixtures thereof.

7. A laundry or cleaning product according to any of Claims 1-6 wherein the bleaching agent is selected from the group consisting of perborate, percarbonate, and mixtures thereof, and preferably also comprising a bleach activator.
8. A laundry or cleaning product according to any of Claims 1-7 wherein the surfactant is selected from the group consisting of anionic surfactants, preferably alkyl sulfate and/or linear alkyl benzene sulfonate surfactants, cationic surfactants, nonionic surfactants, and mixtures thereof.
9. A method for laundering fabrics, said method comprising contacting fabrics in need of cleaning with an aqueous solution containing an effective amount of one or more hexosaminidase enzymes, preferably an aqueous solution of a composition according to any of Claims 1-8.
10. A method for cleaning dishes and tableware, said method comprising contacting dishes or tableware in need of cleaning with an aqueous solution containing an effective amount of one or more hexosaminidase enzymes, preferably an aqueous solution of a composition according to any of Claims 1-8.
11. A method for cleaning dishes and tableware according to Claim 12 wherein said method is carried out in an automatic dishwashing machine.



# INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/US 98/09125

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 6 C11D3/386

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 C11D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 425 019 A (PROCTER & GAMBLE) 2 May 1991 see page 7, line 2 - line 45 see claims 1-6; example 5	1,3-9
X	DATABASE WPI Section Ch, Week 9320 Derwent Publications Ltd., London, GB; Class B04, AN 93-163586 XP002080339 & JP 05 095784 A (NAKANO VINEGARS DEALER KK), 20 April 1993 see abstract	1,3,9-11
A	WO 96 36700 A (NOVONORDISK AS ) 21 November 1996 cited in the application see claims	1,2

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

12 October 1998

Date of mailing of the international search report

22/10/1998

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

Int. l. Application No

PCT/US 98/09125

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		CA 2028560 A,C	28-04-1991
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